Plant Pest Diagnostics Branch

California Department of Food & Agriculture

2001

PLANT PEST DIAGNOSTICS CENTER

Plant Pest Diagnostics Branch

2001 Annual Report

The primary mission of the Plant Pest Diagnostics Branch is to provide timely and accurate plant pest diagnostics in support of the California Department of Food and Agriculture (CDFA) pest prevention program. The Branch also serves as a scientific resource for a number of clients in addition to CDFA, including the United States Department of Agriculture, other federal and state agencies, County Agricultural Commissioners, University of California Cooperative Extension, the agriculture industry, and the public. The scientific and technical staffs contribute to global scientific knowledge in plant pest diagnostics and biosystematics.

This annual report is a summary of the accomplishments completed in the last fiscal year. It highlights critical areas of diagnostics research, new methodology, and updates on ongoing projects. This report is by no means inclusive of all the work performed in the branch but is representative of key results.

The staff of the Plant Pest Diagnostics Branch continues to provide leadership in science and excellence in service.

Dennis E. Mayhew, Ph.D.
Branch Chief

Andrew's Clearwing Moth (*Carmenta andrewsi* Eichlin).
This handsome moth from Baja California, is named for Fred G. Andrews,
recently retired Coleopterist at PPDC.

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Cover: *Marmara gulosa* (the citrus peel miner) mining on (from top) grapefruit, cotton, pepper, and grapes. See report, pages 15-16, *Polyphagy of Citrus Peel Miner*.

November 20, 2001

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Dedication of State Collection of Arthropods (CSCA)

CDFA Meadowview Campus, Sacramento, California

Tuesday, October 15, 2001; 11:35 a.m. to 11:55 a.m.

William (Bill) J. Lyons, Jr., Secretary
California Department of Food and Agriculture

Secretary Bill Lyons (right) presents Dr. Alan Hardy with CSCA plaque.
photo by D. Mayhew

By an act of the State Legislature, the arthropod collections of CDFA, Plant Pest Diagnostics Center, became the official California State Collection of Arthropods (CSCA). On October 16, 2001, the Secretary of the California Department of Food and Agriculture, William (Bill) J. Lyons, Jr., presided over the formal dedication. The text of his remarks follows.

"Thank you Dennis for your kind introduction. I'm pleased to be here today and am grateful for the opportunity to meet so many of you and to see firsthand the outstanding work being done by our department's scientists.

For nearly two centuries, Oktoberfest has brought communities together to celebrate the end of the harvest season and to enjoy the rewards of months of hard work. Today we celebrate not only the bounty of our farms, but also the contributions of the scientific community to making that bounty possible.

Scientific contributions to agriculture are too often overlooked, despite the fact that research is the cornerstone of success for all farmers. Regardless of what or where or how much they produce, farmers depend on your scientific expertise each and every day. You are the behind-the-scenes heroes of agriculture.

Since I became Secretary, it has been my goal to emphasize the importance of science within the department. For this reason, I am proud to have been a part of the department's establishment of the first-ever Office of Agricultural and Environmental Science Advisor, led by Dr. Mimi Sen.

This position was created to emphasize the close relationship between scientific and agricultural advancements, and to provide leadership on scientific policies and endeavors. As you know, these can be overwhelming tasks, but I know that the job is in capable hands with Dr. Sen. Together we will work to keep science a top priority not only in terms of policy but in terms of budgeting as well.

We have much to be proud of in our department. As part of the ongoing effort to provide leadership in science and excellence in service, the scientists in the diagnostics branch have developed a number of world class scientific collections, the most important of which is the insect collection which represents hundreds of man years of scientific endeavor.

Because of the value of this collection as a scientific resource, the legislature approved Assembly Concurrent Resolution 32 authored by Assemblymember Cardoza, establishing the insect collection as the official California State Collection of Arthropods. This designation not only recognizes the collection's scientific value, but also establishes it as the official repository for specimens of California arthropods.

It takes talent, hard work, and dedication to build such a collection. I speak for the department and all of California's agricultural community when I say thank you to all of the entomologists past and present who have contributed to this effort. I especially want to recognize Dr. Fred Andrews, who has served as curator of this collection and has dedicated his career to making this the outstanding resource that it has become.

Unfortunately, Dr. Andrews, who is retiring at the end of the year, is not present today. I would therefore like to present a plaque commemorating this dedication to Dr. Alan Hardy, who will assume duties as curator.

Another thank you is in order. I want to recognize the efforts of the California Association of Professional Scientists who are responsible for shepherding the resolution through the legislative process and for providing the dedication plaque. CAPS is also to be commended for their representation of the department's scientific workforce, and I look forward to continuing a positive working relationship with the association.

In closing, I'd like to thank each and every one of you for your dedication to bringing cutting-edge science to our state's agricultural community. Keep up the good work!"

Interesting Moths Recently Found in California

Thomas D. Eichlin and Scott A. Kinnee

The following records of moths new to California were first reported in the California Plant Pest and Disease Report (CPPDR) (Gill 2000)

An Oleander Moth

In August 2000, pyralid moths (see figure) were reared from oleander (*Nerium oleander*). The larvae were taken at a residence in Newport Beach, Orange County, California. Although the moth is quite striking in appearance, it could not be immediately identified. Mr. Michael Shaffer, British Museum of Natural History, London, responded to queries regarding the moth stating, "It belongs to a species-complex under the name *Glyphodes onychinalis* (Guenee, 1854), subfamily Spilomelinae (Crambidae)." This onychinalis-complex consists of several species (some undescribed) covering the Afro-Asian area, extending through Indonesia, Australia and New Zealand. Mr. Shaffer states that, based on the heavy markings, the California specimens most closely resemble material from New Zealand and almost certainly consists of an undescribed form from this species-complex. He further states that we were correct in assuming this species has been introduced into California, and as far as he is aware is the first American record.

Dr. M. Alma Solis, Systematic Entomology Lab., USDA, National Museum of Natural History, Washington, D.C., concurred with Mr. Shaffer on the identity of the moth. She could find no specimens of this moth in the National Museum collection but saw a large series of species in the "complex" from the Philippines and others from Africa, including South Africa. Dr. Solis agreed with Shaffer saying, "Michael is also correct that it is probably the first record for the U.S." She could find no references to any of these species being reared from oleander or any citations regarding their biology.

In September 2000, one additional adult specimen was forwarded to the PPD Center, which had been captured in an avocado tree at a residence in Coronado, San Diego County. In the absence of feeding evidence or larvae, we do not consider avocado to be a host for this pyralid moth.

An Ash Moth

Recently, September 2000, samples of a small moth were sent to the PPD Center, reportedly defoliating ash trees in Del Norte County. Moths were sent from a golf course in Crescent City and from Oregon ash trees in the Jed. Smith St. Park near Hiouchi. These were identified as *Zelleria* sp. (Yponomeutidae), and based on wing venation, structures of the genitalia and specifics of their behavior, it was determined that it is related to a foreign species, *hepariella* Stainton and not previously recorded from California. *Zelleria hepariella* occurs in Great Britain, through Europe (expanding) and Japan; it is known to feed on ash, producing webbing similar to the unknown species on the leaves.

Firgure 1. Adult of *Zelleria* sp.

The unknown moth is about 10-15 mm in wing spread (Fig. 1). The forewings at rest often appear slightly upturned toward the tips. The larva lives in a thick silken web spun among the leaves; this webbing often includes several larvae. Pupation occurs in a dense white cocoon on the leaf (Fig. 2), often several cocoons formed in the "communal" web. Apparently, the adult over winters in dense cover and probably has one generation per year.

Figure 2. Feeding damage and cocoons on ash leaf.

Two additional species of moths are included in the CPPDR article (p. 20) on new state records: *Pheroeca praecox* (Gozmani & Vari) (Tineidae, plaster bagworm); and *Homaledra sabalella* (Cham.) (Tineidae, palm leaf skeletonizer). Please refer to the article for details.

Reference Cited

Gill, R. J. (*ed.*). 2000. New state records. Calif. Plant Pest & Disease Report, 19(3-6):29-31. Interesting Moths Recently Found in California.

Noctuid Search
An Interactive Key to Identify the Noctuidae of North America

http://cdfa.ca.gov/noctuid/

Thomas D. Eichlin, Dennis E. Mayhew, Scott A. Kinnee, Ronald E. Somerby
(photos by Charles S. Papp and Scott A. Kinnee)

Heliolonche cereris Grote

The Nocutidae is a large family of moths, often difficult to identify. Noctuid Search, an Internet application, provides a search engine to a database containing select species of this family. Though not all-inclusive, the approximately 700 species listed are representative of those most commonly encountered. Emphasis is on the noctuids of California and North America north of Mexico. Moths examined for this database are in the California State Collection of Arthropods, housed in the Plant Pest Diagnostics Center.

The key for identification is interactive and open-ended. By specifying select morphological features of an unknown moth from a set of 21 characters, a short list of probable species is generated. Many of the species' descriptions include a color or black and white photo to aid in identification. Thus, by using obvious key features and figures of the moths, the often-difficult process of determination of noctuid species is made easier.

There has been much favorable comment from several individuals who have used the Noctuid Search program. We continue to update the database, replace black and white photos with new color images and add color figures to the species descriptions.

The nomenclature of each noctuid species and its author was verified using Poole's catalog of the Noctuidae (Poole 1989). The catalog number assigned to each species corresponds to the Check List of the Lepidoptera of America North of Mexico (Hodges, et al., 1983).

References Cited:

Hodges, Ronald W., et al. 1983. Check List of the Lepidoptera of North America North of Mexico. G. W. Classey Limited & The Wedge Entomological Research Foundation, 284 pp.

Poole, Robert W. 1989. Lepidopterorum Catalogus (new series), fasc. 118, Noctuidae (3 parts). E. J. Brill/Flora & Fauna Publications, 1314 pp.

Identification of Oriental Fruit Fly Complex Species Trapped in California

Eric M. Fisher

Bactrocera is the largest genus of fruit flies (Tephritidae), with currently about 500 named species (and more being found and described every year). The vast majority of *Bactrocera* species live in the tropical parts of the Old World (mainly in Asia, the Pacific and Australia; a few are present in Africa). Larvae of *Bactrocera* are primarily fruit inhabiting, and many species are considered to be serious agricultural pests. Over the past 40 years, 16 species of *Bactrocera* have been detected in California; one of these, olive fly (*Bactrocera oleae*), has recently become established here. Although olive fly is monophagous (host specific to olive fruits), many other *Bactrocera* are polyphagous, a trait which enhances their status as pests. Two very major pest species detected in California, both in the Oriental fruit fly (OFF) complex, are highly polyphagous: *Bactrocera dorsalis* infests 153 species of fruit hosts in 46 different families, and *B. papayae* 206 species in 50 families (Allwood, et.al. 1999). Table 1 lists the various species of *Bactrocera* that have been detected in California.

The identification of species in this large genus presents many challenges. From the perspective of the CDFA systematic entomologists, the California *Bactrocera* detections fall into two categories: the OFF group, and the remainder of the genus. For the latter, the identification process is relatively straightforward, as these species usually have distinctive characteristics. In addition, several recent publications (Drew 1989, White & Elson-Harris 1992, White & Hancock 1997) offer great assistance, as they treat the species of Bactrocera of large geographic areas, provide new keys, and collate much information (which previously was very scattered).

The OFF group is much more difficult. Initially it was considered to be a single, widespread, very variable species (*B. dorsalis*); later, a complex of 16 species; and now, following the revision of Drew & Hancock (1994), a complex of 68 species (52 species from the Oriental region, and an additional 16 from the Australasian region). Many of these 68 individual species apparently are sibling species, ones so similar in appearance that they are very difficult to distinguish from one another using morphological characters alone. In fact, the single best identifying characteristic of a sibling species in this complex is the geographic place of origin of each fly. For specimens of these flies found in detection traps in California, this important information is never known.

At the heart of the OFF complex is what might be termed the OFF superspecies. These are three allopatric (geographically distinct) sibling species: *B. dorsalis* (in the strict sense) in north; *B. philippinensis*, in the Philippine Is.; and *B. papayae* in the south (Fig. 1). The three species of the OFF superspecies can be distinguished only by subtle differences in shape and extent of several features (the most important being the apical part of costal wing band, dark markings on dorsum of abdomen and anterior femora, and length of male and female genitalia). As these subtle morphological features vary between individuals of each OFF superspecies, it is usually not possible to positively identify specimens detected in California. Drew (pers. comm.) has stated that he generally needs a minimum of 40 trapped specimens of an OFF complex species before he can make a positive identification.

Several different approaches are now underway to improve our ability to discriminate between members of the OFF complex and OFF superspecies. (1) Molecular: CDFA is funding research on discovering molecular markers that might offer additional diagnostic characters. Collaborators at Kansas State University, Lincoln University (New Zealand), and University of Hawaii are involved. Preliminary results show some utility for markers between various OFF complex species (but not yet between the OFF

superspecies). (2) Field-caught material (native specimens): simultaneous with molecular work are efforts to find representative specimens of every possible OFF complex species from their native habitat; these will provide authenticated voucher specimens for molecular and morphological studies. (3) Specialized training: CDFA systematists have received training from Drew to assist in their use of existing keys and resources; an important facet of this was the opportunity to utilize the very extensive *Bactrocera* collection in Australia. (This country plays a very key role in *Bactrocera* research; they have over 75 years experience in leading this field, and the vast majority of world's *Bactrocera* specimens are preserved in Australian research collections.) (4) Computer-assisted, interactive key: White & Hancock (1997) have produced the very effective CABIKEY to *Bactrocera*, but this key has not worked well within the OFF complex. A CDFA-funded effort to extend this successful concept to OFF is underway by R. Drew and colleagues in Australia. By restricting content to the OFF complex, data for the new interactive key will be based on much larger series of authenticated specimens. Emerging molecular diagnostic information will be incorporated into the key. (5) Curation and dissection: CDFA systematists are preserving California detected OFF specimens for molecular and morphological research. Dissections to analyze details of male and female genitalia are an important component of the curatorial process.

Literature Cited:

Allwood, A., A. Chinajariyawong, R. Drew, E. Hamacek, D. Hancock, C. Hengsawad, J. Jipanin, M. Jirasurat, C. Kong Krong, S. Kritsaneepaiboon, C. Leong and S. Vijaysegaran. 1999. Host Plant Records for Fruit Flies (Diptera: Tephritidae) in South East Asia. The Raffles Bulletin of Zoology. Supplement No. 7: 1-92.

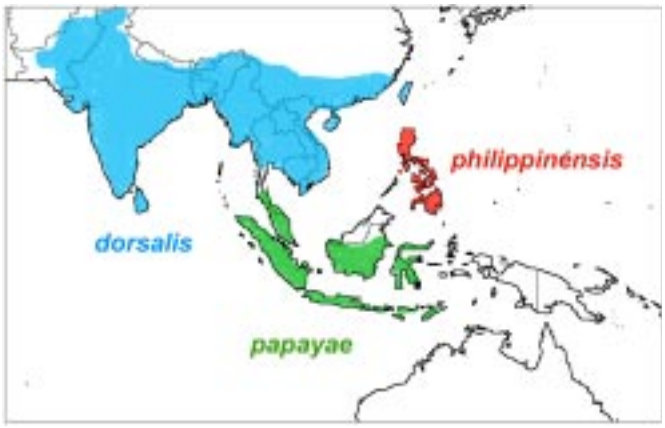
Drew, R. 1989. The tropical fruit flies (Diptera: Tephritidae: Dacinae) of the Australasian and Oceanian regions. Memoirs of the Queensland Museum, 26: 1-521.

Drew, R. and D. Hancock. 1994. The *Bactrocera dorsalis* complex of fruit flies (Diptera: Tephritidae: Dacinae) in Asia. Bulletin of Entomological Research, Supp. 2: 1-68.

White, I. and M. Elson-Harris. 1992. Fruit flies of economic significance; their identification and bionomics. Wallingford: CAB International. xii + 601pp.

White, I and D. Hancock. 1997. CABIKEY to the Indo-Australian Dacini fruit flies. Wallingford: CAB International. CD-ROM.

Fig. 1. Distribution of *Bactrocera dorsalis* superspecies.



B. dorsalis (s. str.) has been accidentally introduced to various islands in the Pacific, including: Guam, Palau, Mauritius, Tahiti, Hawaiian Is., Okinawa, Nauru (eradicated from the last two-mentioned); *B. papayae* has been introduced to Irian Jaya, Papua New Guinea, and Australia (Queensland — since eradicated from there).

Table 1: *Bactrocera* species detected in California.

species	common name	yr detected	remarks
dorsalis complex			
carambolae Drew & Hancock	carambola fruit fly	1997	complete detection status uncertain – due to possible earlier confusion with *dorsalis*
dorsalis (Hendel)	Oriental fruit fly	1960	detected yearly since 1966; most common *dorsalis* complex species in California
irvingiae Drew & Hancock	—-	1998	identification of single female problematical (could be aberrant *dorsalis*)
occipitalis (Bezzi)	Bezzi fruit fly	1983	complete detection status uncertain – due to possible earlier confusion with *dorsalis*
papayae Drew & Hancock	papaya fruit fly	1985	also detected in 1995 and 1998; complete detection status uncertain
philippinensis Drew & Hancock	Philippine fruit fly	1985	also found 1997 and 1998; complete detection status uncertain, probably more common
verbascifoliae Drew & Hancock	—-	1997	three males detected in Diamond Bar (L.A. Co.); identified by D. Hancock & R. Drew
other *Bactrocera*			
correcta (Bezzi)	guava fruit fly	1986	detected frequently since 1986
cucurbitae (Coquillett)	melon fruit fly	1956	detected numerous times, especially since 1985
facialis (Coquillett)	Tonga fruit fly	1998	detected only once
latifrons (Hendel)	solanum fruit fly	1998	detected only once (though found in quarantine situations earlier)
oleae (Gmelin)	olive fruit fly	1998	now widely established in olive areas throughout California
scutellata (Hendel)	—-	1987	single specimens detected in four different years
tryoni (Froggatt)	Queensland fruit fly	1985	another single male detected in 1991
zonata (Saunders)	peach fruit fly	1984	detected frequently since 1984
sp., nr. pallida	—-	1987	single male of undescribed species (R. Drew indicates origin probably India)

Systematics of the Lauxanioidea (Diptera)

Stephen D. Gaimari

My main area of scientific interest is the group of flies know as the Lauxanioidea, which is one of the more primitive acalyptrate superfamilies. The component families have taken two major evolutionary life history pathways. The first ("chamaemyiid-type"), exemplified by Chamaemyiidae [27 genera & subgenera, ~250 described species], is larval predation on plant feeding aphids, adelgids, scales, and mealybugs. The second ("lauxaniid-type"), exemplified by Lauxaniidae [173 genera & subgenera, >1550 species] and Celyphidae [9 genera & subgenera, 78 species], is larval saprophagy in decaying plant matter, likely feeding on associated microorganisms such as fungi, bacteria, and yeasts. The remaining family, Eurychoromyiidae [with only 1 species], currently has unknown feeding habits, although uncovering its life history may be important for understanding certain aspects of lauxanioid evolution.

My current grant-funded research (National Science Foundation) deals with trying to gain a better understanding of the evolution of the Lauxanioidea of the world, laying the ground work for all future studies of this large, important group. The following topics make up this research:

• Place genera into a phylogenetic context, assessing the monophyly of current major subordinate groups.

• Propose a biologically predictive and informative, evolutionary-based classification for genera within the superfamily, specifically revising the current classification of chamaemyiid genera, reassessing the lauxaniid subfamilies Homoneurinae and Lauxaniinae, and reevalutaing the family statuses of the enigmatic Celyphidae and Eurychoromyiidae, which may be subordinate groups within Lauxaniidae.

• Examine patterns of lauxanioid evolution relative to major events in geological history.

• Explore the significance of feeding patterns within the larger groups of Lauxanioidea, particularly those related to the major feeding shift between "chamaemyiid-type" predation and "lauxaniid-type" saprophagy.

• Consider the implications of discovered evolutionary patterns on the evolution of morphological structures.

• Develop a world catalog and on-line database covering all species of the superfamily.

The phylogenetic, evolutionary relationships among genera and subgenera of Lauxanioidea will be elucidated and interpreted using cladistic methodology, thereby testing the monophyly of currently accepted family-group level taxa and genera with multiple subgenera. Using the uncovered phylogenetic patterns, distributional patterns will be examined for corroboration of biogeographical histories, employing component analysis and ancestral area analyses. Phylogenetic patterns will also be utilized in developing a theory for the major feeding shift between larval saprophagy and predation in the early stages of lauxanioid evolution and will be used to examine the more specific host-shift patterns among chamaemyiid genera. Evolution of critical morphological characters will be considered within the context of the phylogenetic hypothesis.

The importance of predictability in a classification cannot be overemphasized, and is necessary for answering fundamental questions in natural history. This predictability is reliant upon sound, biologically relevant definitions for taxa, which can only be achieved through phylogenetic studies at species and

higher levels. A phylogenetically based classification provides insight, for example, into the environmental requirements of predators being considered for biological control, potential host ranges and levels of specificity (from monophagy to polyphagy), and potential distributional ranges and specific habitats for discovering potential natural enemies. Habitat and food requirements can also be highly specific for the saprophagous taxa, and assessing these requirements in the context of a phylogenetic classification can aid in developing conservation strategies within regions with high litter content, such as forest ecosystems.

Additionally, this predictive classification provides a stepping stone towards understanding and realizing the importance of all organisms, whether predacious, saprophagous, or phytophagous, to sound environmental function, highlighting the network of life strategies displayed on the tree of life. Understanding lauxanioid relationships and associated patterns gives us insight into the worldwide radiation of a highly diverse, functionally vital, higher taxon. The relationship between lauxanioid diversification and patterns of historical biogeography will provide insight into the reasons for their present day success, particularly in areas of their greatest diversity, such as Southeast Asia and South America. The nuiances of the feeding shift between saprophagy and predation are fundamental to many monophyletic groups of Diptera and other insects, and provide a step towards understanding the true ecological importance of such groups and how they have evolved.

Invasive Mollusk Conference
CDFA-PPD, Sacramento
June 26-27, 2001

Alan R. Hardy

(jointly sponsored by California Department of Food and Agriculture and
United States Department of Agriculture, APHIS)

Panel members:

David G. Robinson,USDA, PPQ
Robert H. Cowie, University of Hawaii
Robert G. Howells, Texas Parks and Wildlife
James W. Smith, USDA, APHIS

Facilitator:

Alan R. Hardy, California Department of Food and Agriculture

The first day was focused mainly on the Channeled Applesnail, *Pomacea canaliculata,* and its potential as a major pest of rice in California and the United States generally. The format was a series of formal presentations, followed by a round-table discussion, which included those in the audience. From this discussion the findings presented below were derived, and unanimously agreed upon by the panel members.

The second day focused upon pathways and means of introductions of invasive mollusks. From the outcome of this discussion will come a "least wanted" list of mollusks, which could potentially become introduced into the United States. This list will be prepared for the USDA, New Pest Advisory Group, through a cooperative agreement with the American Malacological Society.

Findings of the Invasive Mollusk Conference Concerning *Pomacea canaliculata*

Panel members, in discussion, and with input from the guests in attendance, agreed upon the following:

- *Pomacea canaliculata* and related species is a serious pest of rice in Asia and the Caribbean
- There are three main pathways of introduction
  - -The pet industry
  - -Water garden industry
  - -Movement for human consumption
- Cultural and food preference differences between North America and Asia play a role in snail movements
- Introductions of snails imported by the pet trade pose the greatest risk in North America
- The advent of plastic aquarium plants has permitted the use of more generalist and voracious phytophagous snails
- An attempt should be made to get the voluntary cooperation of the pet industry to remove *Pomacea* species except *brigesii* from the pet trade
- Prevention of infestations is easier and cheaper that eradication later
- Regulation of all snails in the family Ampullariidae may be necessary
- Any regulation should prohibit the transportation, sales or possession of living *Pomacea* species except *brigesii* and their eggs
- Commerce of *Pomacea* snails on the Internet has been increasing
- *Pomacea* snails are sold under a variety of common and scientific names, and there is confusion which names refer to which real species
- A high priority should be made to eradicate existing infestations in Texas and California as soon as possible
- If eradication is impossible, then monitoring and quarantine to prevent further spread is necessary
- Any regulatory action should mandate immediate action upon discovery of infestations
- Immediate action is necessary to prevent spread *of Pomacea canaliculata*
- Education of government, industry and the public is important
- The risk posed by the possible use of *Pomacea canaliculata* as a biological control agent against aquatic weeds outweighs any possible benefit
- Quarantine inspections are best way to prevent accidental or deliberate importation and introductions of *Pomacea*
- Research is needed on:
  - -Rice harvest practices and potential food fouling by snails
  - -Seasonality of snail reproduction
  - -Feeding habits and hosts other than rice
  - -Length of time snail can survive without feeding
  - -Climatic extremes tolerated
- Data are scattered and difficult to compile
- Overseas research on control has been minimal
- The true identity of the pest species (possibly more than one) requires investigation
- There is the need for a formal pest risk assessment, which includes climatic studies. A risk zone map forecasting system developed at North Carolina State University is being considered for this PRA.

Abstracts of Presentations

Invasive Snails: Deliberate Introductions

David G. Robinson (USAD, PPQ, National Mollusk Specialist)

Among the most serious invasive mollusks introduced into the United States are a number of snail species that are intentionally introduced for a variety of reasons. These include several Helicid species, some imported under permit, and others that are smuggled, which are imported for the "escargot" market. In this group, *Cryptomphalus aspersus* (=*Helix aspersa*) or the Brown Garden Snail is the best known, but *Cantareus apertus, Otala lactea, Eobania vermiculata*, and *Helix pomatia* are all now established as a result of accidental or deliberate releases into the environment. There are at least three species of achatinids or "Giant African Snails" that are regularly intercepted by PPQ officers in international travelers' baggage. Live achatinids are imported because of their gustatory and/or medicinal reputation, and for a "pet" market, the extent of which remains unknown. One, *Achatina fulica*, is established in Hawaii, and was introduced into Florida in the late 1960s although successfully eradicated. A third group, aquatic snails belonging to the Ampullariidae (*Pomacea, Marisa* and *Pila* spp.), Viviparidae (*Cipangopaludina* spp.) and Thiaridae (*Melanoides* and *Tarebia* spp.), imported for the aquarium industry and for human consumption, represent the largest number of species of freshwater snails, many of which have been successfully introduced into the environment and now represent a serious threat to agriculture, public health, and the environment.

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Apple Snails as Alien Agricultural Pests in the Asia-Pacific Region, and the Introduction and Spread of Alien Snails in Hawaii and the Pacific Islands

Robert H. Cowie (University of Hawaii)

Apple snails (family Ampullariidae, genus *Pomacea*) were introduced from South America to South-East Asia about 1980. The identity of the species (one or more) involved is not fully clear, though the majority of reports refer to it as *Pomacea canaliculata*. Taxonomic and biogeographic research is needed to resolve the true identity and geographic origins of the species.

Apple snails have since become major pests of rice and other wetland crops in South-East Asia, to the extent that in some countries (e.g., Philippines, Viet Nam) they are now considered the number one rice pest. They continue to spread and huge areas of Asia and Australia are at risk. Four species of apple snails have been reported in Hawaii, though only one (usually referred to as *Pomacea canaliculata*) is a serious agricultural pest, most notably of taro. This species was probably introduced to Hawaii from the Philippines about 1989; it continues to spread rapidly through the Hawaiian Islands. Elsewhere in the Pacific, apple snails have been reported in Guam, Palau, and Papua New Guinea. In North America, alien apple snails (reported as *Pomacea canaliculata*) are now present in Florida, Texas, and California.

Efforts at control of apple snails have involved chemical, biological, and mechanical/cultural methods. None has proven adequately effective. Prevention of the further spread of the snails and rapid eradication of incipient invasive populations are crucial.

Other alien snail species (land and freshwater) continue to invade Hawaii and the Pacific Islands. Of the 90 or so alien snail species that have been introduced to Hawaii, over 30 are established; new introductions are discovered regularly. Through the Pacific, between 100 and 200 alien snail species have been recorded. Most of these are widespread tropical "tramps," readily transported by people. The horticultural industry appears to be especially important in the inadvertent spread of terrestrial species; the pet industry is probably responsible for many of the freshwater introductions. Most of these alien snails

are small (with serious exceptions, such as apple snails and the giant African snail) and introductions often go un-noticed until populations are established in the wild. More effective quarantine and other preventative measures are necessary to halt the spread of these alien species. Public education and political support is key.

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Channeled Applesnail Introductions in Texas, with Comments on Other Exotic Mollusks

Robert G. Howells (Texas Parks and Wildlife)

In past decades, shells of channeled applesnail (*Pomacea canaliculata*) and Florida applesnail (*P. paludosa*) and a population of channeled applesnails (1989-1990) were reported in Texas, but no known applesnail populations were recognized in the state by the late 1990s. However, in July 2000, reproducing populations of channeled applesnails were found in the American Canal and Mustang Bayou between the cities of Houston and Galveston in southeastern Texas. Additional field surveys in late 2000 and early 2001 documented channeled applesnails at a total of nine locations in the Houston area (Galveston Bay drainage) as well as an additional population found near Fort Worth (Trinity River drainage). The Houston area populations are centrally located in the Texas rice belt, a billon dollar a year industry. Because channeled applesnail poses a threat to aquatic habitat and agricultural crops, Texas Parks and Wildlife Department (TPWD) listed it as a harmful or potentially harmful exotic species that is prohibited in Texas. Sale, possession, and transport became illegal as of April 30, 2001. However, to date, no governmental agencies have reported plans to control or eradicate the species in Texas. Indeed, until confirmed agricultural or aquatic habitat damage is documented, control efforts should not be expected. Tropical Storm Allison caused extensive flooding in the Houston area in early June 2001 and probably distributed channeled applesnails to additional canals and bayous. Distribution of this species in Texas may already be beyond readily controllable levels.

Other exotic mollusks have been introduced in Texas as well. These include giant rams-horn snail (*Marisa cornuarietis*), red-rim melania (*Melanoides tuberculatus*), quilted melania (*Tarebia granifera*), marbled aplexa (*Stenophysa marmorata*), tawny aplexa (*S. maugeriae*), Asian clam (*Corbicula fluminea*, and possibly a second species), and edible brown mussel (*Perna perna*). Among the land snails, brown gardensnail (*Cryptomphalus* (=*Helix*) *aspersus*), milk snail (*Otala lactea*), brown-banded snail (*O. vermiculata*), decollate snail (*Rumina decollata*), and rosy wolfsnail (*Euglandina rosea*), also occur in Texas. Of these, only giant rams-horn snail is also listed by TPWD as a prohibited exotic species.

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USDA-APHIS-PPQ New Pest Advisory Group

James W. Smith (USDA, APHIS)

The New Pest Advisory Group (NPAG) is a process within the USDA Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), Center for Plant Science and Technology (CPHST) that has been part of the Plant Epidemiology and Risk Analysis Laboratory (PERAL) in Raleigh, NC, since January 1998. Increasing emphasis on new global markets and world trade has resulted in higher risk for the establishment of exotic plant pests in the U.S.A. In order to protect our trade, agriculture, and ecosystems, the NPAG process was developed for the rapid evaluation and response necessary to mitigate the effects of these introductions. NPAG assess newly detected introductions (arthropods, pathogens, weeds, and mollusks) to determine the recommended course of action. During this evaluation, an ad hoc panel is assembled from Federal, State, and University sources with expertise of the particular pest. The immediate goal is to communicate, document, and ask strategic questions that need to be addressed by PPQ program staff. From the information provided, management decisions are made. NPAG also investigates imminent threats that are established in potential pathways of introduction.

Polyphagy of Citrus Peel Miner

Thomas D. Eichlin and Scott A. Kinnee

As early as 1915, serpentine mining was observed on the peel of citrus fruit in southern California. Though not damaging the internal quality of the fruit, cosmetically the fruit is rendered unacceptable for the market. It wasn't until 2001 that a name was proposed for the moth species causing the unsightly appearance. Throughout this long period, several researchers attempted to rear adults and identify the culprit. All agreed that it was in the genus *Marmara* (Gracillariidae), but no one was confident enough to assign a specific name. Most recently, it was considered by us (by others previously) to be probably *M. salictella* Clemons. Thankfully, a thorough study revealed that it was an undescribed species, and those researchers set the record straight (Guillen et al. 2001). The citrus peel miner is now *Marmara gulosa* Guillen, Davis & Heraty.

This study also showed, using nuclear DNA diagnosis, that the species mining oleander stems adjacent to citrus orchards was essentially genetically the same. Through the efforts of Dennis Haines (Tulare County) and his colleagues and Kris Godfrey and Deborah Mayhew (Integrated Pest Control), many possible hosts for M. gulosa have been discovered, based on similar mining on stems, fruit and leaves; positive identification of the species responsible for the mining on each host plant is still lacking. Dennis compiled a list of all potential host plants, either discovered by himself or as reported to him by others (some damaged hosts are figured here):

Cocoon of *Marmara gulosa*

Crops

Apple – incidental on fruit
Apricot – stems
Avocado – stems
Cherry – stems
Citrus (all varieties) – fruit and stems
Cotton – stems and bolls
Cowpeas – stems and petioles
Eggplant – fruit
Fig – stems and fruit

Crops *cont.*

Grape – stems, petiole, tendril, bunch rachis, berries
Olive – fruit
Peach/Nectarine – stems
Peppers – stems and fruit
Plum – stems and fruit
Pomegranate – fruit
Pumpkin – fruit
Squash – fruit
Walnut – stems and husk
Watermelon – fruit

Ornamentals

Ash (*Fraxinus* sp.) – stems
Chitalpa (*Chilopsis* X *Catalpa*) – stems
English Laurel (*Prunus laurocerasus*) – stems
Euonymus – stems
Grecian Laurel (*Laurus nobilis*) – stems
Hibiscus – stems
Hydrangea – stems
Japanese Maple (*Acer palmatum*) – stems
Mandevilla – stems
Mulberry (*Morus albus*) – stems
Oleander – stems and leaves
Pachysandra – stems
Poplar sp. – stems
Saucer Magnolia (*Magnolia soulangiana*) – stems
Tupelo (*Nyssa sylvtica*) – stems
Willows – stems
Wisteria – stems

Weeds

Amaranthus hybridus (Green Amaranth) – stems
Chenopodium murale (Nettleleaf Goosefoot) – stems
Ipomoea hederacea (Ivyleaf Morningglory) – stems
Ipomoea purpurea (Tall Morningglory) – stems
Nicotiana glauca (Tree Tobacco) – stems
Portulaca oleracea (Purslane) – stems

See cover for mining on (from top) grapefruit, cotton, pepper, and grapes.

Reference Cited:

Guillén, M., D. R. Davis and J. M. Heraty. 2001. Systematics and biology of a new, polyphagous species of *Marmara* (Lepidoptera: Gracillariidae) infesting grapefruit in the southwestern United States. Proc. Entomol. Soc. Wash. 103(3):636-654.

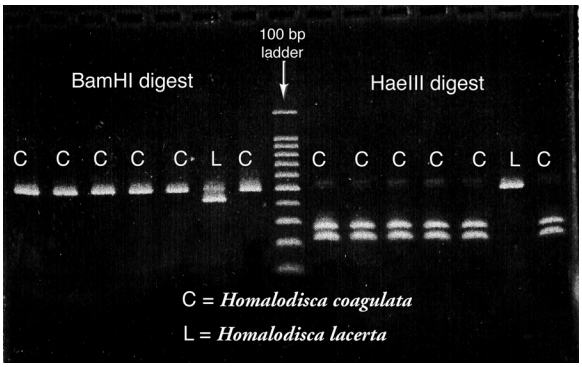
Mitochondrial DNA Differentiaton of Early Life Stages of Two *Homalodisca* Species.

Scott A. Kinnee

Homalodisca coagulata, the glassy winged sharpshooter (GWSS), is native to Mexico and the southeastern United States. Accidentally introduced to California, GWSS is a vector of *Xylella fastidiosa*, the causal bacteria of Pierce's disease.

Although identification of adult sharpshooters is relatively simple, the egg and nymph stages of GWSS are indistinguishable from those of the native smoke tree sharpshooter, *Homalodisca lacerta*. A method of discriminating between these species was required in order to identify the presence of GWSS life stages on nursery stock being moved about the state as well as identifying nascent GWSS infestations.

A polymerase chain reaction-restriction length polymorphism (PCR-RFLP) diagnostic was developed to differentiate between the early life stages of several sharpshooter species present in California, including *H. coagulata* and *H. lacerta*.



Total genomic DNA of adults of *H. coagulata* and *H. lacerta* was extracted. Utilizing the universal primers C1-J-1751 and C1-N-2191 (Simon, et. al. 1994:690), a 497 base pair region of the Cytochrome c Oxidase Subunit I (CO1) of the mitochondrial DNA (mtDNA) was amplified. This PCR product was screened against a range of restriction endonucleases. The digestion products of HaeIII and BamHI proved sufficient to discriminate these species. Later sequencing of this region provided no additional diagnostic restriction sites for separation of these two species.

The samples submitted to the laboratory for identification using this process consist mostly of desiccated egg masses on various host plant materials. The sharpshooter egg tissue is removed from the plant tissue prior to DNA extraction to minimize possible inhibitory compounds from the plant affecting the PCR amplification. Single eggs have sufficient mtDNA present for identification.

Adult *H. coagulata* and *H. lacerta* from different localities continue to be screened for variation within this COI region. A second mitotype has been identified for *H. lacerta*. Thus far, no variation has been found for *H. coagulata* from California within this mtDNA fragment.

Reference cited:

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu and P. Flook. 1994. Evolution, Weighting, and Phylogenetic Utility of Mitochondrial Gene Sequences and a Compilation of Conserved Polymerase Chain Reaction Primers. Ann. Entomol. Soc. Amer. 87(6):651-701.

Revision of the Tribe Eleodini

Ronald E. Somerby

After the publication of Frank Blaisdell's revision of the tribe in 1909, the number of recognized genera where quite stable. His central contribution was the recognition of the significance of the ovipositor's structure in defining the tribe's six genera, which includes the genus *Eleodes*. The shape of the ovipositor's coxite, both its dorsal and ventral perspective, were interpreted as respectively divergent yet significantly stable within each genus. The form of the male aedeagus wasn't perceived as useful. Three of the six genera were monotypic, with the highly evolved genus *Embaphion* having seven species. In contrast, within *Eleodes* over 200 species were recognized, being divided into 13 subgenera. However, most of the species occurred within the nominate subgenus and the subgenus *Blapylis*, accounting for approximately 2/3 of the species.

Seventy years later John Doyen contributed additional insights into the significance of the tribe's ovipositor and male aedeagus (1979). Based upon the highly evolved structure of both the ovipositor and aedeagus within the tribe Eleodini, he determined that the tribe Amphidorini shared the same structural similarities with eleodines, thus placing the tribe's three genera, *Amphidora*, *Cratidus* and *Trichoderulus*, into Eleodini. Today, the three genera are interpreted as only additional species of *Eleodes*, with *Cratidus* being placed in a new subgenus of *Eleodes*. Doyen used the highly complex structure of the aedeagus to show the close affinity of eleodines with at least half of the 15 tribes of Opatrinae.

For the last two years I have investigated the tribe Eleodini, studying both the female ovipositor and male aedeagus in order to develop a better key to the genera within the tribe and the subgenera within the genus *Eleodes*. The earlier keys have not worked in a satisfactory manner. In addition, I have studied the general adult morphology among the many species from a cladistic perspective, a perspective that utilizes apomorphic characters to show sister group relationships. These studies have suggested that nine genera should be recognized for the tribe, with the genus *Eleodes* having 10 subgenera. The most primitive genera are *Lariverius* and *Trogloderus*. Then, two of the subgenera in *Eleodes*, *Tricheleodes* and *Pseudeleodes*, were raised to generic level as the genus *Tricheleodes*, which now includes eight species. Next, the following three genera are interpreted as closely related, with *Discogenia* being taken as the more primitive member. Formerly, the two species of *Discogenia* were interpreted as representing a subgenus of *Eleodes*. The more derived genera are *Neobaphion* and *Embaphion*, with three and eight species, respectively. The more advanced genus *Eleodes* appears to be closely related to the ancestors of *Discogenia*. Finally, the last two, *Eleodimorpha* and *Cratidus*, appear to be specialized closely related genera, with *Cratidus* being formerly a subgenus of *Eleodes*.

The subgenera to be recognized within *Eleodes* are listed from most primitive to advanced: *Blapylis*, 45 species; *Metablapylis*, 4 species; *Caverneleodes*, 3 species; *Ardeleodes*, 1 species; *Heteropromus*, 1 species; *Stenelodes*, 14 species; *Eleodes*, 33 species; *Litheleodes*, 9 species; *Promus*, 13 species, and *Melaneleodes*, 11 species.

References Cited:

Blaisdell, F.E. 1909. A monographic revision of the coleoptera belonging to the tenebrionide Eleodiini inhabiting the United States, lower California, and adjacent islands. Smithsonian Institution, U.S. National Museum, Bulletin 63, pp. 524.

Doyen, J.T. and J.F. Lawrence 1979. Relationships and higher classification of some Tenebrionidae and Zopheridae (Coleoptera). Systematic Entomology, 4, 333-377.

Chrysomelidae Literature
An On-line Reference Source

<http://www.coleopsoc.org/nwslttrs.shtml>

Terry N. Seeno

The newsletter CHRYSOMELA is published by the Plant Pest Diagnostics Center as both an information source on the current activities within the leaf beetle community and as a medium for PPDC literature exchanges with other libraries, museums, and universities. This newsletters has been in print for over 20 years and has been issued, on the average, biannually since October 1979. It is currently being published and has 41 issues to date.

A bibliography of the current, worldwide literature on the Chrysomelidae is a regular feature of the newsletter which appears in every issue. Because the literature on leaf beetles is so expansive, these citations focus mainly on biosystematic references but also contains items on chrysomelid biology, distribution, and host records. Citations were taken directly from the publication, reprint, or author's notes and not copied from other bibliographies.

This bibliographic collection contains 2,635 citations. It has been reassembled in an electronic format and is available only on the internet. Revisions and additions are currently in progress and will be numbered sequentially. Adobe Acrobat® 4.0 was used to distill the list into a PDF file, which is searchable using standard Acrobat search procedures for information regarding the title, author, journal, and date of publication.

The file is currently posted on The Coleopterists Society website under the heading of CHRYSOMELA. The printed document is about 81 pages in length using a 10 point, *Times* or *Times Roman* typeface.

(Sample text:)

Abdullah, M. and A. Abdullah. 1968. *Phyllobrotica decorata* DuPortei, a new sub-species of the Galerucinae (Coleoptera: Chrysomelidae) with a review of the species of *Phyllobrotica* in the Lyman Museum Collection. Entomol. Mon. Mag. 104(1244-1246):4-9, 32 figs.

Abdullah, M. and A. Abdullah. 1969. Abnormal elytra, wings and other structures in a female *Trirhabda virgata* (Chrysomelidae) with a summary of similar teratological observations in the Coleoptera. Dtsch. Entomol. Z. 16(4-5):405-409, 7 figs., 1 tab.

Abdullah, M. and S. S. Qureshi. 1968. *Ashrafia anwarullahi*, A New Genus and Species of the Galerucinae (Coleoptera: Chrysomelidae) from West Pakistan. Pakistan J. Sci. Ind. Res. 11(4):425-426.

Abdullah, M. and S. S. Qureshi. 1968. The Chrysomelidae, Coleoptera of Pakistan. Part III. - A Key to the Genera and Species of the Galerucinae, with Descriptions of New Genera and Species. Pakistan J. Sci. Ind. Res. 11(4):396-414.

Abdullah, M. and S. S. Qureshi. 1968. A New Genus and Species of the Galerucinae (Coleoptera: Chrysomelidae) from Pear Tree in West Pakistan. Pakistan J. Sci. Ind. Res. 11(4):423-424.

Abdullah, M. and S. S. Qureshi. 1969. A key to the Pakistani genera and species of the Chrysomelinae and Halticinae (Coleoptera: Chrysomelidae), with description of new genera and species including the economic importance. Pakistan J. Sci. Ind. Res. 12:105-120, 7 figs., 2 tabs.

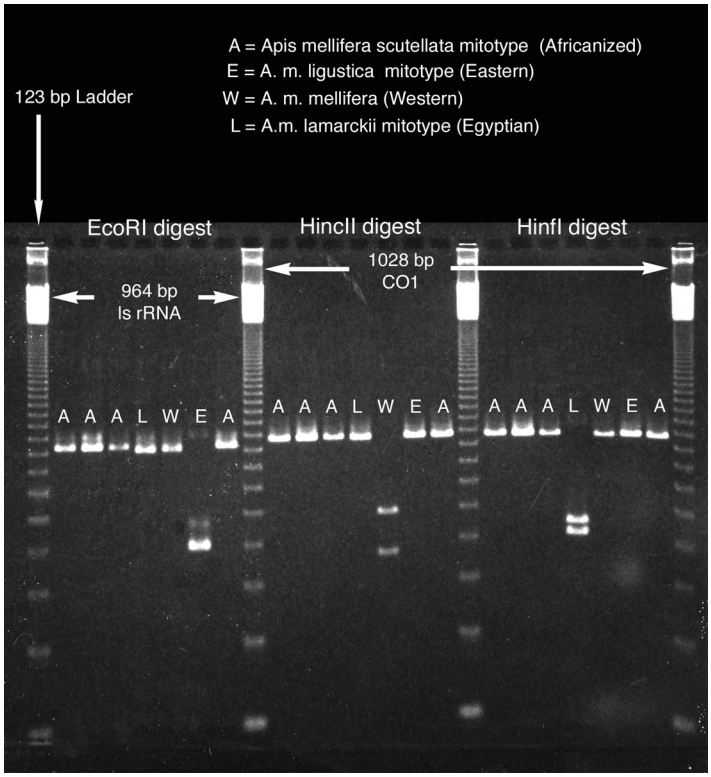
Abdullah, M. and S. S. Qureshi. 1969. A key to the Pakistani genera and species of the Hispinae and Cassidinae (Coleoptera: Chrysomelidae), with description of new species from West Pakistan including the economic importance. Pakistan J. Sci. Ind. Res. 12:95-104, 1 fig., 1 tab.

Adair, R. J. and J. K. Scott. 1991. Distribution, life history, host specificity and suitability of an undescribed *Chrysolina* (Coleoptera: Chrysomelidae) for the biological control of *Chrysanthemoides monilifera* (Compositae). Bull. Entomol. Res. 81:235-242.

Africanized Honeybee Stinging Incidents in California Verified Using mtDNA Markers.

Scott A. Kinnee

The first Africanized honeybees in California were found in Blythe, Riverside County, in October of 1994. The area in California now considered colonized by the AHB is over 51,000 square miles. As the range of the AHB has grown, there has been increased contact with areas populated by humans and domestic animals. This has resulted in a rising number of stinging incidents over the past four years that are directly attributable to the AHB. Africanized honeybees are determined using PCR amplified mitochondrial DNA markers.



Origin of the Sudden Oak Death (SOD) Pathogen and Its Potential Impact: a Working Hypothesis

J. T. Sorensen, F. Hrusa and T. Tyler

Upon the discovery of Sudden Oak Death disease in California in 1995, CDFA became involved in determining whether the pathogen involved was newly introduced into the state, both to determine a course of action for the agency and to assess what might happen as the disease manifested itself. At PPDC in 2000, after assessing the current data available, scientists proposed and published (Sorensen et al. 2000) a hypothesis that may explain the origin of the pathogen causing Sudden Oak Death (SOD) and its evolutionary-ecological factors.

Under the hypothosis' assumed scenario, the previously undescribed *Phytophthora* (since described as *Phytophora ramorum*) implicated in this oak disease, may have coevolved with *Rhododendron* species in the Oriental/Himalayan region. The fungus was then probably brought to Europe and then probably to the Pacific Northwest of North America before arriving in California in 1995, through *Rhododendron* movement. In California, the pathogen infects red oaks (*Quercus agrifolia*, *Quercus kelloggii*) and Tanoak (*Lithocarpus*), which have little resistance and are severely affected, showing quick mortality. White oaks (*Quercus* section *Quercus*) do not appear to be affected in Europe or California and may have developed resistance through historical Asiatic contact. Intermediate oaks (*Quercus* section *Protobalanus*) are also unaffected. The fact that another red oak, *Quercus wislizenii*, has thus far not been affected may indicate that climatic factors, such as cool moist conditions similar to those in the area where the pathogen may have originated, may limit transmissibility. If so, the ultimate distribution of the disease would be limited to susceptible species growing in coastal, summer fog areas. However, if the pathogen can be transmitted in warm-moist conditions, red oak species in the southeastern U.S. may be at risk. Several suggestions for testing this hypothesis and its scenario were presented. This working hypothesis employed assumptions and principles based on coevolution of exploitive agents (parasites, pathogens) and their hosts, as well as biogeographic vicariance of the hosts.

Sorensen et al. (2000) hypothesized susceptible California section Lobatae oaks and *Lithocarpus* eventually should be impacted by the pathogen throughout their range, wherever coastal summer fog or moist sea breeze impacts the local climate. Such areas climatically favor *Rhododendron*. This should be south to the Santa Barbara area. Presumably, this would also involve Oregon populations of *Quercus kelloggii* and *Lithocarpus densiflorus*. It is uncertain if the winter tule fog conditions of the California Central Valley might involve *Quercus wislizeni* at low elevations in that region. Presumably *Quercus kelloggii* populations in the Sierra Nevada (Mt. Lassen and south) occur at high enough elevations that they do not commonly contact tule fog; its populations in the northern Central Valley may extend low enough to contact tule fog.

Sorensen et al. (2000) state it is uncertain if non-Californian section Lobatae oaks might be affected by the pathogen, but a common Madro-Tertiary history and phylogenetic lineage might predispose those oaks in the southern and southeastern U.S. to susceptibility. It is uncertain if the pathogen in the southern U.S. might be favored by warm humid conditions. However, an Anthracnose pathogen on *Cornus florida* in the eastern U.S. requires warm-moist conditions; that pathogen has been introduced into California for at least a decade, in the northwest corner of the state, yet has failed to move out of the initial area, presumably due to its tight requirements for the local cool-moist climate in that part of California. If the SOD pathogen accepts the warm-moist conditions of the southern U.S., section Lobatae oaks in that region may be at risk. Unfortunately, adequate assessments of the phylogenetic relationships among the genera of Fagaceae are lacking, but other Nearctic genera in the family include: *Chrysolepis*, *Castanea*,

and *Fagus*. It is uncertain how, or if, these will be affected. Reviewing Judd and Kron (1993), for the phylogenetic relationships among the Ericaceae, to which *Rhododendron* belong, Sorensen et al (2000) suggested the following genera may also be at risk for infection: *Kalmia*, *Kalmiopsis*, *Phyllodoce*, *Ledum*, *Arbutus*, *Arctostaphylos*, *Erica*, and *Vaccinium*.

Sorensen et al. (2000) suggested the following procedures to test the Judd and Kron hypothosis:

1. Confirm, through nucleotide sequencing and Koch's postulates, that the *Rhododendron* pathogen and the oak pathogen are the same, exhibiting cross-transmissibility.

2. Confirm the identity of the *Rhododendron* pathogen as the same as that in Europe. Check for its occurrence in the Pacific Northwest.

3. Confirm that the pathogen cannot infect Californian section Quercus oaks, or at least does not develop the virulence noted on section Lobatae oaks.

4. Check infestation areas in California to determine if cultivated *Rhododendron* is present and infected, and if dispersion patterns indicate the potential of transmission to *Quercus*.

5. Check transmissibility to section Lobatae oaks that occur in the southern and southeastern U.S. and Mexico.

6. Check resistance of Oriental *Lithocarpus* species. Check resistance of Palearctic section Quercus oaks.

7. Check for climatically restrictive parameters in transmissibility requirements to section Lobatae oaks, especially with regard to atmospheric moisture and temperature regimes: specifically, whether: (1) cool-moist conditions are necessary for transmissibility, (2) warm-moist regimes support transmissibility, and (3) if winter tule fog conditions support transmissibility.

8. Check susceptibility among *Rhododendron* (including "azalea") species to determine the susceptibility throughout the genus, particularly those species and populations native to northern California and the PNW. Check susceptibility in the following genera: *Kalmia*, *Kalmiopsis*, *Phyllodoce*, *Ledum*, *Arbutus*, *Arctostaphylos*, *Erica* and *Vaccinium*.

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Judd, W.S. & K.A. Kron. 1993. Circumscription of Ericaceae (Ericales) as determined by preliminary cladistic analyses based on morphological, anatomical, and embryological features. Brittonia, 45 (2): 99-114.

Sorensen, J.T., F. Hrusa & T. Tyler. 2000. Origin of the Sudden Oak Death (SOD) pathogen and its potential impact: a working hypothesis. Calif. Plant Pest & Disease Report, 19 (3-6): 49-57.

Testing Seeds for Plant Parasitic Nematodes:
an Improved Method for Nematode Extraction and Analysis

John J. Chitambar

Certain species of plant parasitic nematodes are able to contaminate seeds by internally infesting developing seeds, externally infesting surrounding plant tissue such as, seed husk and glumes, or by being associated with plant debris in seed lots. Plant parasitic nematode species that infest seeds are feeders of above ground parts of plants and have developed the capability to withstand dry environmental conditions by entering a biological state of dormancy during their life cycle. Hence, infested seed provide a primary means for the survival and widespread dispersal of nematode pathogens. Furthermore, not all infested plants exhibit apparent symptoms of disease even if their seeds may be infested with nematodes (McGawley et al. 1984, Gergon & Mew 1991). These biological facts are a major cause of concern for many countries that import seed from California and have established stringent phytosanitary quarantine requirements against certain unwanted plant parasitic nematode species associated with seed (Table 2.).

Over the past several years, the dramatic increase in world trade of seed has brought about an increased need for biologically based phytosanitary standards and corresponding certification tests for pests and pathogens of concern. Official tests are needed and often required by the governments of importing countries. Presence of any number of unwanted target nematode pests in a sample can result in the non-issuance of federal phytosanitary certificates and preclusion of commodity shipments to foreign markets.

In order to develop a standard seed-nematode extraction test that could be used in official certification laboratories and meet federal standardization and accreditation rules (7CFR 353), Griesbach and Chitambar, et al. (1999) conducted a comparative analysis of four extraction methods using as a model the Seed Gall Nematode, *Anguina agrostis* (an internally seed-borne nematode species), from infested grass seed samples. These methods were developed and analyzed at the testing laboratories of the Oregon Department of Agriculture, Plant Division and the California Department of Food and Agriculture (CDFA), Nematology Laboratory and included: 1) *Water agar blend method*: Seeds presoaked and aerated in water were germinated on water agar plates, blended with the medium, sieved and centrifuged; 2) *Sieve-blend method*: Presoaked and aerated seeds were blended and sieved; 3) *Misting method*: Presoaked and aerated seeds were placed under water mist; 4) *Blender-funnel-host stimulant method*: Seeds were blended, then placed over a funnel containing aerated water, and 0.1 g plant host leaf tissue. Method 4 proved to be the best method for extracting live nematodes from seed and was recommended by the authors.

The Nematology Laboratory, CDFA, adopted the method recommended by Griesbach and Chitambar, et al. (1999). Certain adaptations were deemed necessary to accommodate bulk regulatory samples, as well as the biology of the nematode species targeted for extraction while maintaining the basic principle of the method.

Method

Under the current modified method, a 10 to 50 g dry seed sample is placed in a high-speed blender with 150 ml distilled water and blended for 45 seconds. The mixture is then poured through 850-µm-pore-size (20 mesh) and 25-µm-pore-size (500 mesh) sieves. The sieve residues are then placed on two layers of tissue draped on a 850-µm-pore-sized stainless steel screen, which, depending on the target nematode species, is placed either on a large funnel containing water or in a funnel and tube apparatus in an intermittent mist chamber. After 24 to 48 hours, nematodes are extracted from the water in the tube or funnel by passing the suspension through a 500-mesh sieve and concentrating the resulting nematode population in a small volume (~20 ml) of water.

Results and Discussion

The method has been used successfully over the past few years at the Nematology Laboratory, CDFA. Table 1 presents a qualitative list of plant parasitic nematode species extracted from infested seed samples of various plant hosts submitted to the Nematology Laboratory, CDFA.

The modifications referred to in the above method excluded host leaf tissue. This was done mainly due to the practical unavailability of host leaf tissue with bulk regulatory samples, and as Griesbach and Chitambar, et al. (1999) did not find any significant difference from numbers of nematodes extracted using leaf tissue alone, without aeration. The choice of placing a blended seed sample with or without mist depended on the nematode species. The White-tip of Rice Nematode, *Aphelenchoides besseyi*, is an aggressively motile nematode that may take from several minutes to hours to settle undisturbed at the bottom of a tube or funnel stem. A sample containing *A. besseyi* would most likely result in the nematodes being washed away in the overflowing currents of water from the tube of a funnel-tube apparatus under mist. The nematodes would be better extracted in an undisturbed, consistent volume of water contained in a funnel not misted. *A. besseyi* was extracted from all the water in the funnel after 24 hours to account for the limited amounts of oxygen present. Longer durations have resulted in rapid deterioration of the sample and nematodes in suspension. On the other hand, the Stem and Bulb Nematode, *Ditylenchus dipsaci*, is a less motile nematode than *A. besseyi* and not affected by overflowing currents of water within a tube under mist. Moreover, intermittent mist also provided fresh supplies of oxygenated water, allowing a longer period of moist incubation while enhancing reactivation and extraction of the nematode.

The level of precision necessary for regulatory nematology requires the optimization of any nematode extraction method and warrants periodic reevaluations of traditional and new methods used in diagnostic analyses. The work discussed in this article is a step towards that goal, and my research continues as well in other areas of methodologies in regulatory nematology.

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Gergon, E. B. & T. W. Mew. 1991. Evaluation of methods for detecting the nematode *Aphelenchoides besseyi* Christie in routine seed testing of rice. Seed Science and Technology, 19:647-654.

Griesbach, J. A., J. J. Chitambar, M. J. Hamerlynck & E. O. Duarte. 1999. A comparative analysis of extraction methods for the recovery of *Anguina* sp. from grass seed samples. Journal of Nematology, 31:635-640.

McGawley, E. C., M. C. Rush & J. P. Hollis. 1984. Occurrence of *Aphelenchoides besseyi* in Louisiana rice seed and its interaction with *Sclerotium oryzae* in selected cultivars. Journal of Nematology, 16:65-68.

Table 1. Plant parasitic nematodes detected in seed samples at the Nematology Laboratory, CDFA, using the described seed-nematode extraction method. 1998-200

Nematode species	Seed host	Detection program	Yr detected
Aphelenchoides besseyi	Rice (paddy)	Phytosanitary quarantine (export)	1999 2001
Ditylenchus dipsaci	Fava bean Beet Pea Alfalfa Rice (paddy)	Phytosanitary quarantine (export)	1998 1999 1999 1999 2001
Ditylenchus sp.	Carrot	Phytosanitary quarantine (export)	1999

Table 2. Partial list of countries with phytosanitary quarantine restrictions against plant parasitic nematode species in seed shipments from California.

Country	Crop	Nematode species of concern	Nematode-seed relationship[1]
Argentina	Rice (seed)	*Aphelenchoides besseyi*	ESB
Brazil	Rice (seed) Sorghum, Clover	*A. besseyi* *Ditylenchus dipsaci*	 ISB
Chile	Orchard grass Foxtail, Oats Rice (seed)	*Anguina agrostis* *D. dipsaci* *Aphelenchoides besseyi*	ISB ISB ESB
Colombia	Alfalfa Oats Orchard grass Rice (seed)	*D. dipsaci* *Anguina tritici* *Bidera mani, A. agrostis* *Aphelenchoides besseyi*	ISB ISB C, ISB ESB
European Union	Alfalfa Rice (seed)	*D. dipsaci* *Aphelenchoides besseyi*	ISB ESB
Federated States Micronesia	Potato	*Globodera pallida*[2]	C
Hong Kong	Rice (plant/seed)	*A. besseyi*	ESB
Inter-African Group	Rice (paddy) Soybean	*A. besseyi* *Heterodera glycines*[2]	ESB C
Malaysia	Rice (seed) Potato	*A. besseyi* *G. rostochiensis*[2]	ESB C
Mexico	Alfalfa Soybean	*D. dipsaci* *H. glycines*[2]	ISB C
New Caledonia	Rice (seed)	*A. besseyi*	ESB
Poland	Alfalfa	*D. dipsaci*	ISB
Saudi Arabia	Wheat	All plant parasitic nematodes	
Sri Lanka	Beet	*H. schactii*	C
Taiwan	Onion, Shallot	*D. dipsaci*	ISB
Turkey	Rice (paddy/seed) Soybean	*A. besseyi* *H. glycines*[2]	ESB C
Uraguay	Rice (seed)	*A. besseyi* *D. angustus*[2]	ESB ISB

[1]ESB = externally seed borne; ISB = internally seed borne; C = seed debris contaminant as nematode cysts, found in plant roots and soil.
[2]Nematode species not known to be present in California.

Collected Data for DNA-based Identifications of Root-Knot Nematodes (*Meloidogyne* spp.)

Ke Dong

Root-knot nematodes (*Meloidogyne* spp.) are major pests of a wide range of crops. Most of the *Meloidogyne* species have broad host ranges and some have more than one host race. Control, management and regulation of these nematodes require accurate diagnosis. Classical methods of identification based on morphology, cytogenetics and differential host range tests have been effective and applied for decades. Electrophoretic enzyme phenotypes have also proven to be a practical means for identifying species and/or sub-species of *Meloidogyne*. The utility of isozyme phenotypes for common *Meloidogyne* species identification has been accepted in most of the root-knot nematode research projects. For example, *M. arenaria, M. incognita, M. javanica, M. chitwoodi,* and *M. naasi* can be distinguished via esterase phenotypes. In addition to the isozyme phenotypes, differences in 2-D protein patterns have proven useful for separating species and infra-species or variants of *Meloidogyne*. Monoclonal and/or polyclonal antibodies have been developed for *Meloidogyne* spp. This technology has great potential for use in regulatory as well as diagnostic and management programs.

While morphology continues as the primary focus in root-knot nematode identification, nucleic acid analyses offer much for the future. These emerging approaches to assessment and characterization of nematode species and populations offer much for advancing our understanding of nematode biodiversity, general ecology, and population assessments for management and regulatory purposes. The range of techniques now available for studying DNA has resulted in much progress in characterizing genomic, mitochondria, and ribosomal DNA of nematodes. In addition to advancing our understanding of nematode phylogenetic relationships, this rapidly expanding information also provides useful tools for nematode diagnostics. DNA based methods for *Meloidogyne* spp. identification could be more objective and reliable than traditional systems. Also, molecular probes may be suitable for any life stage present in a sample. The DNA techniques developed and utilized in the diagnostic purposes include: amplified fragment length polymorphisms (AFLP); specific oligonucleotide probe for southern-blot and dot-blot; microsatellite and repetitive DNA; polymerase chain reaction (PCR) and multiplexed PCR; randomly amplified polymorphic DNA (RAPD); restriction fragment length polymorphisms (RFLP); ribosomal DNA (rDNA) intergenic (IGS) and internal transcribed (ITS) spacer regions; sequence; sequence characterized amplified region (SCAR); sequence tag site (STS); and variable number tandem repeat (VNTR). For molecular diagnostic assessments, the objective is to obtain low-cost, user-friendly and reliable molecular data that distinguishes nematode species or sub-species levels. Table 1 is a brief summary of current available information for root-knot nematode species, sub-species and population identifications. Based on the ongoing molecular characterization of nematode-plant interactions, the above approaches may prove fruitful for species and population-level assessments of these important crop pathogens.

Table 1. Summary of DNA analyses of root-knot nematodes (*Meloidogyne* spp.) *.

DNA fragments/probes (RFLP/Southern-Blot/Dot-Blot etc.)

1.	Genomic RFLP (*Meloidogyne* spp.)	Curran et al. 1985. Parasitology 90:137-144.
2.	Genomic DNA fragments (*Ma*, *Mh* raceA/B, *Mi*, and *Mj*)	Curran et al. 1986. JON 18:83-86.
3.	Total mtDNA probe and RFLP (*Ma*, *Mh*, *Mi*, and *Mj*)	Powers et al. 1986. JON 18:288-293.
4.	rDNA probe (*Mh* races A and B)	Curran and Webster 1987. Canadian J of PP 9:162-166.
5.	Total mtDNA variations (*Ma*, *Mh*, *Mi*, and *Mj*)	Powers and Sandall 1988. JON 20:505-511.
6.	Genomic DNA complexity (*Ma*, *Mh*, *Mi* and *Mj*)	Pableo and Triantaphyllou. 1989. JON 21:260-263.

7.	Total mtDNA probe (*Mc*, and *Mh*)	Hyman 1990. JON 22:24-30.
8.	Total mtDNA probe (*Mc*)	Hyman et al. 1990. JON 22:273-278.
9.	mtDNA fragment probe (*Ma*, *Mi* and *Mj*)	Harris et al. 1990. JON 22:518-524.
10.	Repeated genomic DNA (*Mi* pop.)	Castaghon-Senero et al. 1991. JON 23:316-320.
11.	Specific oligonucleotide DNA (*Mi* pop.)	Chacon et al. 1991. Parasitology 103:315-319.
12.	RFLP/Southern Blot (*Meloidogyne* spp./pop.)	Garate et al. 1991. JON 23:414-420.
13.	Repetitive genomic DNA probe (*M. artiellia*)	De Giorgi et al. 1991. Nematol. Medit 19:131-134.
14.	Random genomic DNA (*Ma* pop.)	Carpenter et al. 1992. JON 24:23-28.
15.	RFLP/Southern Blot (*Mi*)	Xue et al. 1992. F&A Nematology 15:35-41.
16.	Repeated DNA probes (*Ma*, *Mh*, *Mi*, *Mj* and *M. hispanica*)	Piotte et al. 1992. F&A Nematology 15:271-276.
17.	mtDNA probe (*Ma*, *Mh*, *Mi*, and *Mj*)	Cenis et al. 1992. Phytopathology 82:527-531.
18.	Non-radioactive probe (*Mi*)	Chacon et al. 1993. F&A Nematology 16:495-499.
19.	mtDNA probe (*Mh* pop.)	Peloquin et al. 1993. JON 25:239-243.
20.	Repetitive DNA (*Meloidogyne* spp.)	Castaghon-Senero et al. 1993. Heredity 70:195-204.
21.	Satellite DNA (*Mh* pop. and *Mi* pop.)	Piotte et al. 1994. Gene 138:175-180.
22.	Genomic DNA probe (*Ma*)	Baum et al. 1994. Phytopathology 84:489-494.
23.	Satellite DNA probe (*Mh* pop.)	Piotte et al. 1995. Phytopathology 85:458-462.
24.	RFLP (*Ma*, *Mh*, *Mi*, *Mj* and *M. mayaguensis*)	Fargette et al. 1996. F&A Nematology 19:193-200.
25.	Satellite DNA (*Mc*)	Castagnone-Sereno et al. 1998. J. Mol. Evol. 46:225.
26.	Satellite DNA (*Mc*, *Mf*, and *Mh*)	Castagnone-Sereno et al. 1998. Mol. Bio. Evol. 15:1115.
27.	Non-radioactive satellite DNA probe (*Mc* and *Mf*)	Castagnone-Sereno et al. 1999. Phytopathology 89:380.
28.	Repetitive DNA probe (*Mh* pop.)	Dong et al. 2001. Nematropica 31:17-23.

PCR/PCR-RFLP/RAPD-PCR/SCAR

mtDNA:

1. TAAATCAATCTGTTAGTGAA
   ATAAACCAGTATTTCAAACT (*Ma*, *Mh*, *Mi* and *Mj*) — Harris et al. 1990. JON 22:518-524.
   Cenis et al. 1992. Phytopathology 82:527-531.
   Stanton et al. 1997. F&A Nematology 20:261-268.

2. GGTCAATGTTCAGAAATTTGTGG
   TACCTTTGACCAATCACGCT (*Ma*, *Mc*. *Mh*. *Mi* and *Mj*) — Powers and Harris 1993. JON 25:1-6.

3. ATCGGGGTTTAATAATGGG
   AAATTCAATTGAAATTAATAGC — Hugall et al. 1994. Genetics 136:903-912.

4. GAAATTGCTTTATTGTTACTAAG
   TAGCCACAGCAAAATAGTTTTC
   TGAATTTTTTATTGTGATTAA
   GAAAAATAAAAAAATTTTGTT
   ATGATTTTTTGTGTCTGCTCA
   AATTTCTAAAGACTTTTCTTAGT
   TTTCCCAACCTATTAAAACCTCT
   AATCTGCTCCATTTAACT (*Moloidogyne* spp.) — Stanton et al. 1997. F&A Nematology 20:261-268.

5. TCACGTATTTTTTTTAGAAGAACAGTC
   AATAATAAATGTTTTTTAAATTCTTTGAGA (*Ma*)
   CTTTTATGCCTATTATAATTGAGGTTG *(Mc)* — Sui et al. 2001. JON 33:
   TGGTCGATCATATGAATATAGGGAT (*Mh*)
   GCGTGAT TGGTCAAAGGTAGCGAGA (*Mi*)
   GTAATTTTTATAGTTGTGATATTGTTATAT (*Mj*) — Sui et al. 2001. JON 33:

rDNA:

a. Cistron (fragment):

TCCCTTAGTAACGGCGAGTG
TGTACAAAGGGCAGGGACG (*Mc*, *Mf* and *Mh*) — Petersen and Vrian 1996. F&A Nematology 19:601-605.

Table 1. *Continued*

b. IGS region:

1. CTGAACGCCTCTAAGTTAGAATCT GCGCTGACAGAATCAATCAG (*Meloidogyne* spp.)	Ellis et al. 1986. Nucleic Acid R 14:2345-2364.
2. TTCGAGTAAGCGCGGGTAAACG GTGAACACCACTCTCATC **or** CAGTTCAGGCAGGATCAAC (*Mc*, *Mf*, and *Mh*)	Petersen and Vrian 1996. F&A Nematology 19:601-605.
3. AGCCAAAACAGCGACCGTCTAC GATCTATGGCAGATGGTATGGA (*Mc*) TGGGTAGTGGTCCCACTCTG (*Mf*)	Petersen et al. 1997. F&A Nematology 20:619-626.
4. TTAACTTGCCAGATCGGACG TCTAATGAGCCGTACGC (*Ma*, *Mh*, *Mi*, *Mj* and *M. mayaguensis*)	Blok et al. 1997. JON 29:16-22.

c. ITS region:

1. GTTTCCGTAGGTGAACCTGC ATATGCTTAAGTTCAGCGGGT (*Meloidogyne* spp.)	Chambers et al. 1986. Gene 44: 159-164.
2. TCATTACGTCCCTGCCCTTTG TTTCACTCGCCGTTACTAAGG	Vrain et al. 1992. F&A Nematology 15:563-573.
(*Mc*, *Mh*, *Mi* and *Mj*)	Zijlstra et al. 1995. Phytopathology 85:1231-1237.
(*Ma*, *Mc*, *Mh*, *Mi*, and *Mj*)	Power et al. 1997. JON 29:441-450.
(*Mc*, *Mf*, *Mh*, and *Mi*)	Zijlstra et al. 1997. F&A Nematology 20:59-63.
(*Mc*, *Mf*, *Mh*, and *Mi*)	Zijlstra 1997. F&A Nematology 20:505-511.
(*Mc* and *Mf*)	Petersen et al. 1997. F&A Nematology 20:619-626.
3. CGTAACAAGGTAGCTGTAG TCCTCCGCTAAATGATATG (*Mc*, *Mh*, *Mi* and *Mj*)	Ferris, et al. 1993. F&A Nematology 16:177-184. Zijlstra et al. 1995. Phytopathology 85:1231-1237.
4. CGCCCGTCGCTGC GGGTGATCTCGACTGAGC (SCAR for *Mh*) CCTTTGTACACACCGCCCGTCGG AGCCTAGTGATCCACCGATAAGGA (SCAR for *Mj*)	Sui et al. 2001. JON 33:
5. TCCTCCGCTAAATGATATG CTTGGAGACTGTTGATC (*Mh*) GAATTATACGCACAATT (*Mc*, and *Mf*) TGTAGGACTCTTTAATG (*Mi*) (SCARs for multiplex PCR)	Ferris, et al. 1993. F&A Nematology 16:177-184. Zijlstra 1997. F&A Nematology 20:505-511.
6. TTGATTACGTCCCTGCCCTTT GTAGGTGAACCTGCAGATGGAT ACGAGCCGAGTGATCCACCG (*Ma*, *Mc*, *Mh*, *Mi*, *Mj*)	Powers et al. 1997. JON 29:441-450.

d. Genomic:

1. RAPD-PCR (*Ma*, *Mh*, *Mi*, and *Mj*)	Cenis. 1993. Phytopathology 83:76-80.
2. AFLP-PCR (*Meloidogyne* spp.)	Xue et al. 1993. F&A Nematology 16:481-487.
3. Random 8-mer PCR (*Ma*, *Mh*, *Mi*, and *Mj*)	Baum et al. 1994. MPMI 7:39-47.
4. RAPD-PCR (*Meloidogyne* spp/pop)	Castagnone-Sereno et al. 1994. Genome 37:904-909.
5. RAPD-PCR (*Ma*, *Mh*, *Mi*, and *Mj*)	Guirao et al. 1995. Phytopathology 85:547-551.
6. Satellite DNA PCR (*Mh*)	Castagnone-Sereno et al. 1995. Current Gen 28:566-570.
7. RAPD-PCR (*Ma*, *Mh*, *Mi*, *Mj* and *M. mayaguensis*)	Blok et al. 1997. F&A Nematology 20:127-133.
8. RAPD-PCR (*Mj* pop.)	Carneiro et al. 1998. F&A Nematology 21:319-326.

Table 1. *Continued*

9. CAGGCCCTTCCAGCTAAAGA CTTCGTTGGGGAACTGAAGA (SCAR for *Mh*) CCAATGATAGAGATAGGCAC CTGGCTTCCTCTTGTCCAAA (SCAR for *Mc*)	Williamson et al. 1997. JON 29:9-15.
10. CTTGTTCGATTCACCTCTTCATCCTC CTATGAAATTCAAAAATC (Satellite Primers for *Mc*) AGAATGAAGATTTTTTGAAG AGCTTCATTATATTCAGAAT (Satellite Primers for *Mf*) GAGTTCCTTGTAAAGCAACTTCT GGACTTTTTTAGGACACAGC (Satellite Primers for *Mh*)	Castagnone-Sereno et al. 1999. Phytopathology 89:380.
11. CCAAACTATCGTAATGCATTATT GGACACAGTAATTCATGAGCTAG (SCAR for *Mf*) TGACGGCGGTGAGTGCGA TGACGGCGGTACCTCATAG (SCAR for *Mh*) TGGAGAGCAGCAGGAGAAAGA GGTCTGAGTGAGGACAAGAGTA (SCAR for *Mc*)	Zijlstra. 2000. European J of PP 106:283-290.
12. TCGGCGATAGAGGTAAATGAC TCGGCGATAGACACTACAACT (SCAR for *Ma*) CTCTGCCCAATGAGCTGTCC CTCTGCCCTCACATTAAG (SCAR for *Mi*) GGTGCGCGATTGAACTGAGC CAGGCCCTTCAGTGGAACTATAC (SCAR for *Mj*)	Zijlstra et al. 2000. Nematology 2:847-853.
13. TCGAGGGCATCTAATAAAGG GGGCTGAATATTCAAAGGAA (SCAR for *Ma*) GGCTGAGCATAGTAGATGATGTT ACCCATTAAAGAGGAGTTTTGC (SCAR for *Mh*) TAGGCAGTAGGTTGTCGGG CAGATATCTCTGCATTGGTGC (SCAR for *Mi*) CCTTAATGTCAACACTAGAGCC GGCCTTAACCGACAATTAGA (SCAR for *Mj*)	Dong and Lewis. 2001. Nematropica 31:
14. GATAGAG*TCGAGGGCATCTAATAAAGG* GGTTGG*GGGCTGAATATTCAAAGGAA* (SCAR for *Ma*) GGGT*TAGGCAGTAGGTTGTCGGG* GAGA*CAGATATCTCTGCATTGGTG* (SCAR for *Mi*)	Sui et al. 2001. JON 33:
15. CCATTTCTGCTAAATGCCAAACTA GGACACAGTAATTCATGAGCTAG (*Mf*) TGACGGCGGTGAGTGCGA TGACGGCGGTACCTCATAG(*Mh*) GGCATTGACGTGCTCCGAGAGT GGTCTGAGTGAGGACAAGAGTA(*Mc*) (SCARs for multiplex PCR)	Zijlstra. 2000. European J of PP 106:283-290 Zijlstra. 2000. European J of PP 106:283-290
16. CGCCCGTCGCTGC GGGTGATCTCGACTGAGC (*Mh*) CCTTTGTACACACCGCCCGTCGG AGCCTAGTGATCCACCGATAAGGA (*Mj*) GATAGAGTCGAGGGCATCTAATAAAGG GGTTGGGGGCTGAATATTCAAAGGAA (*Ma*) GGGTTAGGCAGTAGGTTGTCGGG GAGACAGATATCTCTGCATTGGTG (*Mi*) (SCARs for multiplex PCR)	Sui et al. 2001. JON 33:

Table 1. *Continued*

SEQUENCE/AFLPs

1.	rDNA sequence (*Ma*)	Vahidi et al. 1988. J of Mol. Evol. 27:222-227.
2.	rDNA sequence (*Ma*)	Vahidi et al. 1991. Gene 108:281.
3.	IGS repeat and sub-repeat regions (Ma)	Vahidi et al. 1991. M&G Genetics 227:334-336.
4.	mtDNA sequences and repeated regions (*Mj*)	Okimoto et al. 1991. Nucleic Acid R 19:1619-1626.
5.	Satellite DNA sequences (*Mh* pop. and *Mi* pop.)	Piotte et al. 1994. Gene 138:175-180.
6.	mtDNA sequence divergence (*Mi*)	Powers et al. 1993. JON 25:564-572.
7.	IGS sequences (*Ma*, *Mh*, *Mi*, *Mj*, and *M. mayaguensis*)	Blok et al. 1997. JON 29:16-22.
8.	ITS sequences (*Ma*, *Mc*, *Mh*, *Mi* and *Mj*)	Powers et al. 1997. JON 29:441-450.
9.	mtDNA sequences (*Mh*)	Hugall et al. 1997. Mol. Biol. Evol. 14:40-48.
10.	mtDNA sequences (*Ma*, *Mc*, *Mh*, *Mi*, *Mj* and *M. hispanica*)	Stanton et al. 1997. F&A Nematology 20:261-268.
11.	ITS sequences (*Mc*, *Mf*, *Mh*, and *Mi*)	Zijlstra 1997. F&A Nematology 20:505-511.
12.	AFLP fingerprinting (*Ma*, *Mi*, and *Mj*; spp/pop)	Semblat et al. 1998. Mol. Ecology 7:119-125.
13.	AFLP (*Ma*, *Mc*, *Mf*, *Mh*, *Mi* and *Mj*)	Van der Beek et al. 1998. F&A Nematology 21:401-411.

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* Abbreviations: *Meloidogyne arenaria* (*Ma*); *M. chitwoodi* (*Mc*); *M. fallax* (*Mf*); *M. hapla* (*Mh*); *M. incognita* (*Mi*); *M. javanica* (*Mj*).

Amplified fragment length polymorphisms (AFLP); mitochondrial deoxyribonucleic acid (mtDNA); polymerase chain reaction (PCR); randomly amplified polymorphic DNA (RAPD); restriction fragment length polymorphisms (RFLP); ribosomal DNA (rDNA); intergenic (IGS) and internal transcribed (ITS) ribosomal spacer regions; sequence characterized amplified region (SCAR); Comparisons were made among nematode species (Spp) and/or populations (Pop).

Annual Report of the Nematology Sample Processing Laboratory: Facts, Figures and New Finds

René Luna and John J. Chitambar

The Nematology Laboratory of the Plant Pest Diagnostics Branch comprises three Nematologists, one Agricultural Biological Technician and a support staff of four temporary employees. During the fiscal year, 2000-'01, new hires to the laboratory staff included the technician and three seasonal employees. The technician heads the team of support staff primarily responsible for the sample processing and data management functions of the laboratory.

Handling Nematode Samples

Samples are routinely collected and sent to the Nematology Laboratory by County Agricultural and State personnel. These samples are designated to either Quarantine, Nursery, Commercial, Dooryard (residential) or Other Zoological programs, and are sent as non-processed "raw' samples, or as processed samples of preserved nematode suspensions in vials. Approximately six counties possess nematode sample processing facilities and personnel trained and certified by the State Nematology Laboratory. Plant parasitic nematodes being microscopic, inhabit above and below ground plant parts as well as the soil around plant roots, depending on the species and biology of the nematode involved. Hence, samples comprised of the plant and/or soil media are potentially inhabited by plant parasitic nematodes. The State laboratory uses a combination of several scientific tests or procedures to extract nematodes from their infested media or samples. Each of these procedures involves the use of large volumes of water, as nematodes are essentially aquatic animals requiring moisture for activity. The number of tests involved in extracting and preparing a collection of nematodes in clear water suspension for diagnostic evaluation emphasize the fact that the workload of the Nematology Sample Processing Laboratory cannot be measured solely by sample load.

Nematode Detection Program	No.of samples
Quarantine (total)	**5,495**
- Incoming External Quarantine	4,552
- Export Phytosanitary Certification	826
- Border stations and ports	78
- Other	39
Nursery (total)	**2,099**
- Registration and Certification	1,452
- Nematode Control (Certification)	643
- Other	4
Commercial	**836**
Dooryard/Residential	**10**
Other Zoological Identifications	**28**
Total	**8,590**

Table 1. Total numbers of samples per program received by the Nematology Laboratory in FY 2000-'01

During 2000-'01, a total of 8,590 nematode samples were diagnosed at the Laboratory. Of these, a total of 4,037 processed samples and 4,454 non-processed samples were received. A breakdown of sample type per program is presented in Table 1. The bulk of quarantine samples include those entering the State through the External Quarantine for Burrowing and Reniform Nematodes program and those exported to other countries through the Quarantine Phytosanitary Certification program. The former are comprised of mainly indoor decorative foliage plants sold at nurseries, while the latter consists of mainly plant seeds processed and examined for targeted nematode species not wanted by importing countries. Most nursery samples of plants for sale by the grower comprised garlic (592 samples), strawberries (920 samples), grape (598 samples) and stone fruit plant parts and root zone soil collected through the State's Registration and Certification, and

Nematode Control programs. In addition, the Nematology Laboratory assisted the State of Nevada Department of Agriculture in diagnosing all their garlic samples for certification.

The numbers in Table 2 reflect the nematode detection program's recent activity, which varies among counties, as it is influenced by such factors as geographic location, number of nurseries, county laboratory facilities, etc.

County	No. of Samples	County	No. of Samples
San Joaquin	1,869	Yolo	68
San Mateo	891	Yuba	52
San Diego	785	Butte	45
Alameda	435	Lake	45
Shasta	335	Kern	43
San Luis Obispo	302	Santa Cruz	29
Madera	283	Sonoma	21
Los Angeles	252	Tulare	20
Mono	248	Modoc	18
Merced	207	Humboldt	17
Sutter	206	Stanislaus	12
Imperial	185	San Francisco	11
Santa Barbara	148	Contra Costa	10
Glenn	147	Ventura	10
Lassen	147	Orange	5
Solano	129	Placer	3
Fresno	127	Kings	3
Sacramento	115	Mendocino	2
Siskiyou	108	San Benito	2
Riverside	98	Calaveras	1
Monterey	91	Del Norte	1
Colusa	77	Napa	1
Tehama	75	Santa Clara	1

Table 2. Total number of samples submitted per County to Nematology Laboratory, FY 2000-2001

Data Management

The Laboratory staff maintains all information on nematode samples in a DBASE III+ database program. The database has about 30 fields of information obtained from Pest and Damage (PDR) forms, including the final diagnoses made by the respective nematologists. State, County and University personnel on request used information from the database during the year.

A new database was introduced by the State for maintaining data records and enabling the electronic transmissions of PDR information between the State Laboratory and county offices. This database will eventually replace the old version.

New and Important Nematode Species Detected

From July 2000 to October 2001, eight "A" rated nematode species, and three "Q" rated nematode species were detected in quarantine samples, while seven "B" rated nematode species were detected in

commercial samples. Totals of 362 "C" rated, and 746 "D" rated nematode species were detected in 8,590 nematode samples examined during July 2000 to June 2001.

Table 3 lists new and important finds of plant parasitic nematodes to California during the past year. Among these were: The Burrowing Nematode (*Radopholus similis*), the Reniform Nematode (*Rotylenchulus reniformis*), the Awl Nematode (*Dolichodorus heterocephalus*), and the Dagger Nematode (*Xiphinema setariae*), all "A" rated pests not present in the California, detected in plants entering the State from infested areas, and of major economic importance to California's Agricultural industry. The Columbia Root-knot Nematode (*Meloidogyne chitwoodi*), and the California Dagger Nematode (*Xiphinema index*) are "B" rated pests of limited distribution within the State. The White-tip of Rice Nematode (*Aphelenchoides besseyi*) although an "A" rated pest, is suspected to be of limited distribution and occurrence in cultivated paddy acreage within California. A Pin Nematode (*Gracilacus latescens*) was detected in great numbers in incoming quarantine plants. Although the Laboratory detected this species for the first time, its presence or economic damage potential to California agriculture is unknown.

Nematode species	Common name	County	Detection Program	Associated Host
Aphelenchoides besseyi	White-tip of Rice Nematode	Sutter	Quarantine Phytosanitary	*Oryza sativa* (Paddy rice)
Dolichodorus heterocephalus	Cobb's Awl Nematode	San Mateo	Incoming External Quarantine	*Ficus benjamina* (Ornamental)
Gracilacus latescens	Pin Nematode	Orange	Incoming External Quarantine	*Phyllostachys bambusoides* (Bamboo)
Meloidogyne chitwoodi	Columbia Root-knot Nematode	Modoc	Commercial	*Solanum tuberosum* (Potato)
Radopholus similis	Burrowing Nematode	San Diego	Incoming External Quarantine	*Phoenix roebelenii* *Rhapis excelsa* (Ornamentals)
Rotylenchulus reniformis	Reniform Nematode	San Diego	Incoming External Quarantine	*Anthurium* sp. *Dypsis lutescens* (Ornamentals)
Xiphinema index	California Dagger Nematode	Tulare	Commercial	*Vitis vinifera* (Grape)
Xiphinema setariae	Dagger Nematode	Sacramento	Incoming External Quarantine	*Olea europaea* (Black Olive)

Table 3. New and important finds of plant parasitic nematodes not present, unknown, or of limited distribution in California. Nematology Laboratory Detection July 2000 to October 2001

Nematode Regulatory Diagnostics and Molecular Genetics

Robert W. Hackney

For many years there has been a strong desire among research scientists (i.e., academic, corporate and regulatory) to test the reliability of molecular genetics for identifications by comparing diagnostics using molecular techniques against diagnostics using classical morphometrics.

The University of California's Division of Agriculture and Natural Resources (DANR) funded "Nematology, (http://danr.ucop.edu/_progcouncil/list_ratified_workgroups.cfm) Diagnosis and Management of Lesion Nematode, *Pratylenchus* sp., in a Methyl Bromide Free World." The basic observation is that a critical need exists for identifying the presence of *Pratylenchus vulnus* and *P. penetrans* in samples from perennial crops. *Pratylenchus vulnus* and/or *P. penetrans* can occur as components (i.e., main effects) within mixed populations of other taxa that are not necessarily damaging to perennials. Quite often pest management decisions are made with less than 10 specimens that have been recovered from any of the following: a soil sample; a root sample; or a sample consisting of roots and soil.

CDFA has the lead responsibility for determining whether fruit and nut tree rootstocks and grapevines from nurseries harbor pertinent taxa. In all scenarios involving identifications/diagnostics to be used for regulatory purposes (and even many research programs), an incorrect identification or one made too slowly for meaningful regulatory action can have severely negative consequences in California's agriculture.

Twelve species of *Pratylenchus* spp. have been identified and reported from California (Siddiqui, Sher and French 1973). Six (or more) of those are regarded as major economic pests. Although there can be an overlap of host ranges, specific taxa are specific to important crops (i.e., perennial vine and tree crops such as grape, walnut, peach and almond).

The current regulatory diagnostics of *Pratylenchus* spp. is based mainly upon classical morphometrics. Recently PCR of the rDNA ITS regions has been shown to be reliable for identifying *Pratylenchus agilis*, *P. bolivianus*, *P. brachyurus*, *P. coffeae*, *P. crenatus*, *P. fallax*, *P. goodeyi*, *P. loosi*, *P. mediterraneus*, *P. neglectus*, *P. penetrans*, *P. pratensis*, *P. pseudocoffeae*, *P. scribneri*, *P. subranjani*, *P. thornei*, *P. vulnus* and *P. zeae* (Waeyenberge, et al, 2000). This diagnostic method is currently operational at the University of California, Davis. Because Waeyenberge's list of *Pratylenchus* spp. includes taxa occurring in California and intercepted in shipments into California, we plan to participate in testing to determine if Waeyenberge's tests will be acceptable for performing CDFA's routine regulatory diagnostics of *Pratylenchus* spp. The University of California, Riverside has an extensive collection of frozen samples containing numerous species of *Pratylenchus* for research at the morphological level (i.e., morphometrics) of identification. A major objective is to develop species-specific primers (i.e., forward and reverse primers) that will yield PCR products with species-specific fragments of different sizes for each population/collection of *Pratylenchus* sp. tested. PCR will be performed upon a single nematode, and the value of multiplex PCR and microplex PCR will be tested.

Recently, by using improved diagnostic techniques, *Arabis* mosaic virus was found in a grapevine rootstock variety at the University of California's Foundation Plant Material Services foundation block. Apparently the infection was not detected when the variety was initially introduced, but cuttings have been distributed from those vines for about 12 years.

I established a morphometrics/morphological laboratory standard for the regulatory diagnostics/identification of *Xiphinema* spp. that will correctly identify the potential vector(s) of *Arabis* mosaic virus

in California's viticulture. Based upon current literature, formal presentations at the California Nematology Workshop and unpublished personal communication, the Nematology Laboratory/Program now uses the revised polytomous key for the identification of *Xiphinema* spp. by P.A.A. Loof and M. Luc (1997).

Working with the other CDFA Nematologists, we established a laboratory standard for extracting *Xiphinema* spp. from field samples presumed (based upon field history, cropping history and information from the nursery stock certification program) to be infested by those taxa. Specific criteria for performing the Nematology Laboratory's regulatory diagnostics of *Xiphinema* spp. using peer-reviewed, published values for pertinent classical morphological characters were evaluated and adopted by the Nematologists. Timely and accurate identifications in this group of nematodes are especially important, because of their established virus vector relationship(s) with grapevine and the nepoviruses (viz. *Arabis* mosaic).

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Non-Native Vascular Plants Occurring Spontaneously in California Beyond Those Addressed in *The Jepson Manual*
Part I –Abbreviated

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Abstract

A catalogue of **315** non-native vascular plant taxa documented as occurring spontaneously in California beyond those addressed in *The Jepson Manual: Higher Plants of California* has been compiled. The catalogue of species is currently in pre-publication review and is not presented here; it was developed from new collections by the authors and others, previously existing herbarium specimens, formal publications, other printed reports, and direct communications with field botanists. Only reports backed by herbarium vouchers are accepted as adequately documented. Of the **315** species, **51** are fully or sparingly naturalized in relatively undisturbed natural habitats, **61** are established in disturbed areas, **27** are tenuously established or locally persisting, **37** are presumed to be non-persisting casuals (waifs), **19** are non-escaped weeds of greenhouse or similarly cultivated environments, **4** have likely been extirpated, and the current status of **116** is unknown. In addition, **12** reported species are here specifically excluded as based on erroneous information. Species highlighted as already being fully established, in some cases achieving pest status, are *Amaranthus rudis, Brassica fruticulosa, Boehmeria cylindrica, Calystegia silvatica* ssp. *disjuncta, Cabomba caroliniana, Cotoneaster lacteus, Crataegus monogyna, Dittrichia graveolens, Fumaria capreolata, Geranium purpureum, Geranium rotundifolium, Hedera canariensis, Limnobium laevigatum, Maytenus boaria, Pyracantha crenatoserrata, Salvinia molesta, Trifolium tomentosum, Trifolium vesiculosum,* and *Verbascum olympicum.* The relatively recent appearance of so many non-native plants, many with a high potential for causing serious infestations, serves to underscore just how much the weed invasion of California is continuing unabated.

Introduction

The significance of invasive non-native plants has recently gained prominence, as evidence mounts for both the environmental and economic devastation such invasions can cause. A recent issue of *BioScience* (51[2], February 2001), for example, is devoted to the topic of "Global Movements of Invasive Plants and Fungi." On the national level, various legislative initiatives have been proposed to address the problem, such as the Harmful Nonnative Weed Control Act (S. 198). At the local level, Weed Management Areas, which coordinate the efforts of public and private agencies and organizations, now blanket most of California.

Obviously, for all of these efforts to work properly, accurate and comprehensive information needs to exist on which non-native plants occur within the area of concern and what potential level of threat they represent. While the average citizen might assume that this information is readily available, especially in the Internet age, the reality is unfortunately otherwise. This is primarily because, although a broad spectrum of professional biologists and amateur enthusiasts eagerly hunt down and keep track of rare native species, non-natives have been historically under-reported if not outright ignored. As a result, our existing knowledge of the identity, occurrence frequency, and distribution of non-native plants is often sketchy or preliminary.

California is by no means an exception to this rule, and the incomplete coverage of non-native species was one of the acknowledged short-comings of *The Jepson Manual: Higher Plants of California* (Hickman 1993), which otherwise represented the state-of-the-art coverage of California plants at the time of its publication. In a subsequent statistical analysis of the numbers and distribution of the non-native taxa reported in the *Manual*, (Rejmanek & Randall 1994), mention was made of some clearly naturalized plants that were missing, but no compilation was attempted. As a precursor tally by Hrusa and Ertter, over 70 non-native plant taxa beyond those included in the *Manual* were noted as naturalized in California (Ertter, 2000). Moreover, the composition, frequency and distribution of plants in a given area are not static; these are usual features of the non-native component, but even for native plants, frequent updates to floristic accounts are necessary to maintain currency (Yatskievych & Raveill 2001). This catalogue is the first installment of a comprehensive effort to follow through on this preliminary note by compiling existing reports and documenting new occurrences of plant taxa that occur spontaneously in California but which were not treated in *The Jepson Manual.*

Significance of New Reports

The often pestiferous nature of the most prominent weeds in California is well-recognized by the layman and professional alike (e.g., Bossard et al. 2000) What is not so universally understood is that these widespread and/or noxious pests began their occupation of California as seemingly innocuous roadside waifs, occasional garden volunteers, minor seed source contaminants, or other localized populations, remaining for some time in non-prominent situations. The California Dept. of Food and Agriculture (CDFA) rates noxious weeds and applies control efforts accordingly, not by their current affect on agricultural economics, but by their potential effect; the State takes the position that control is both most biologically and cost effective when the populations are small, before the plant has become a widespread, established pest. Data have been assembled and published in support of this position (Bayer 1999). Thus, the treatment of only the most widespread and well-established weeds in California by *The Jepson Manual – Higher Plants of California* (Hickman 1993) leaves a gap in our knowledge that may affect the future of California's agricultural activities, pristine wildlands and outdoor recreation areas. That this is an important gap may be recognized when one considers that every presently widespread weed in California began long ago as a "casual" [*Centaurea solstitialis* (yellow starthistle)*, Lepidium latifolium* (perennial pepperweed)], or "occasional garden escape" [*Cytisus scoparius* (Scotch broom)*, Genista monspessulana* (French broom)].

While it cannot be claimed that every plant appearing in this catalogue will ultimately become even a local pest, the establishment of any non-native plant may ultimately have long-lasting impacts, and even currently casual taxa have this potential, because, unlike many kinds of environmental pollutants that are eventually neutralized within or disappear from the system upon termination of the source, biological pollutants such as naturalizing non-native plants are self-perpetuating, often increasing in prominence and distribution with time, regardless of whether the source has been terminated (O'Kennon et al. 1999). Moreover, as stated above, the relationship between the size of a weed infestation at control initiation and the cost in dollars for control is logarithmic, with cost increasing exponentially according to increasing infestation size (Bayer op. cit., Rejmanek 2000). As a result, awareness of the identity and ecology of a potential or incipient weed population is not only important, it is also economically imperative — if control or eradication are eventual goals.

We hope that this compilation will spur two activities: first, further exploration for non-native spontaneous plants in California; and second, clarification of the current status of the taxa on this list, especially those classified as "unknown." Clearly, the disturbed areas, on which some of these latter taxa established in the earlier decades of this century, have subsequently been stabilized by development; however, other populations may have expanded their range but are now on private land, no longer readily accessible.

On-going Need for Vouchered Reports

The present compilation is not exhaustive for several reasons. First and foremost is the rapid rate at which new weedy taxa are being found in a state as large and geographically diverse as California. Secondly, a compilation such as this, which includes many records known only from obscure locations or single sites in California, comprises a major effort by botanical collectors of modern and previous eras; without their past and ongoing efforts, this collation would not exist. With this in mind, it is important, as California's increase in human population and consequent plant introductions continues unabated, that the collecting and documenting of non-native taxa continue also.

As a final note, we wish to emphasize the absolute need for vouchered documentation of new reports of spontaneous non-native plants (see Dean and Hrusa 2000 for instructions on collecting and documenting plant occurrences). The fact that determining and verifying these species does not keep pace with the introduction rate is not only a frightening statement as to the future of California's plant life, but a reflection of the difficulty in applying names to plants whose geographic origins are not known and which may be cultivated forms or which have become phenetically modified in their new habitats. For this reason it has been discouraging to realize how many seemingly authoritative reports, via both agencies and private organizations, lack confirming specimen material. Indeed, some lacked even the documentation of who applied the name and when, or where the plant was reported to grow. Without such information a reliable understanding of which taxon is actually represented is not possible. The ramifications of this information gap are great; ecological behavior, environmental tolerances, developmental and phenological patterns are often species-specific, and control measures, whether physical, chemical, or biological, may not be effective if the totality of life history is not correctly understood. The essential first step toward accessing whatever critical information is available is having an accurate identification.

The relatively recent appearance of so many non-native pest plants with a high potential for causing serious infestations further underscores just how much the weed invasion of California is continuing unabated. Thus, the current catalogue, while the result of intensive research in both the field and herbarium, is by no means the last word, but rather serves to point out the extent to which new non-native plants must be constantly sought out and reported (preferably before achieving pest status).

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Table 1: The relationship of species in the current catalogue to *The Jepson Manual* (Hickman 1993).

130	Discovered prior the *Manual* but either not identified or a report not published.
110	Discovered (published report or not) subsequent to the *Manual*.
62	Discovered and a report published prior to the *Manual*.
11	Re-determination of a pre-*Manual* species.
2	Accidentally excluded from the *Manual*, but treatment completed.

Table 2: List of taxa not in *The Jepson Manual*, highlighted as fully established in California.

Amaranthus rudis	Well-established in widely separated localities.
Brassica fruticulosa	Rapidly spreading in mostly disturbed areas.
Boehmeria cylindrica	Currently expanding wetland invader.
Calystegia silvatica ssp. *disjuncta*	Widespread in coastal areas, previously misidentified.
Cabomba caroliniana	Rapidly expanding, well-established aquatic invader. Dominant in some Sacramento River delta locations.
Cotoneaster lacteus	Becoming locally more common, expected more widely.
Crataegus monogyna	Appearing in widely separated regions, often in stable habitats.
Dittrichia graveolens	Rapidly expanding throughout the San Francisco Bay area; recently reported also from San Diego Co.
Fumaria capreolata	Unrecognized, probably more common than previously thought, likely expanding.
Geranium purpureum	Rapidly becoming more abundant.
Geranium rotundifolium	Widespread, overlooked previously, or rapidly expanding.
Hedera canariensis	Persistent invader of stable forest understories; previously confused with or included in *H. helix*.
Limnobium laevigatum	Rapid colonizer of open, still water at low elevations. Can dominate to the exclusion of all other aquatic species.
Maytenus boaria	Aggressive invader of burns, forming long-lasting seedbanks.
Pyracantha crenatoserrata	Widespread; invader of mesic shorelines.
Salvinia molesta	Recent aquatic introduction into California where spreading rapidly in the Colorado River and canal system.
Trifolium tomentosum	Widespread; previously overlooked or misidentified.
Trifolium vesiculosum	Recent introduction found in widely separated localities, suggesting potential for rapid establishment.
Verbascum olympicum	Well-established in natural habitat, expanding for more than 20 years.

Photo: G.F. Hrusa, 1998.

Figure 1: *Ononis alopecuroides* (foxtail restharrow). Pasture infestation in San Luis Obispo County. Only known site in North America for this Eurasian species.

Photo: G.F. Hrusa, 1998.

Figure 2: *Boehmeria cylindrica* (bog hemp). Georgiana Slough in the Sacramento River delta. Also known from Snodgrass Slough (both Sacramento Co.) it has been observed all the way downstream as far as Rio Vista. Native to eastern North America it has been accidentally introduced into several western states. These are the first records for California.

Photo: G.F. Hrusa, 2000.

Figure 3: *Verbascum olympicum* (Anatolian mullein). Leslie Creek in Sonoma County where this species has been spreading downstream steadily since 1976. Introduced to a local garden as an ornamental from where it escaped. Only known site in North America. Native to Eurasia.

Current Curation Activities in The California Department of Food and Agriculture Seed Herbarium

J. Chesi

The California Department of Food and Agriculture seed herbarium was established in 1922. At that time, it contained 15,000 specimens. The collection now contains over 58,000 specimens and is the third largest in the world. Included are seed herbaria from Beecher Crampton (U.C. Davis), the Mojave Desert Study (U.S. Department of Agriculture), the Wildlife Investigations Laboratory (California Department Fish and Game), Los Angeles County Seed Laboratory and Sacramento Federal Seed laboratory. New specimens continue to be added at a rate of approximately 1,000 per year.

Fig. 1: Herbarium cabinets, each cabinet contains 40 herbarium boxes.

Fig. 2: Herbarium box, each box contains 100 vials.

At present, a continuing effort is being made to develop and update an herbarium database detailing collection data for all specimens, while concurrently confirming identifications and updating nomenclature. Anomalies are compared with specimens at the California Academy of Science (CAS) and CDFA Plant Herbarium (CDA). Specimens that cannot be reconciled are either removed or noted in the database. The Beecher Crampton collection and the family Asteraceae are currently completed and available in electronic form.

The Effect of Seed Orientation and the Bound Primary Root Condition in Pepper (*Capsicum* spp.) Seedlings.

Paul S. Peterson

Seed coat adherence is frequently observed in testing pepper seed. The coat-bound condition of pepper cotyledons has been described by Baker (1948) and is attributed to the inflexible seed coat in combination to less than optimum environmental moisture conditions after radicle emergence.

Seed coat adherence at the base of the seedling hypocotyl causing bound primary and secondary roots is also observed in seed testing laboratories. In agriculture, a seed is considered as germinable only if essential seedling structures are present and "normal." A well-developed root system is one of these criteria. For years, the bound root condition in seed tests has been considered a seedling defect. In the CDFA laboratory, this condition has been studied and is caused by the seed orientation in the germination test (Peterson 1988, Peterson & Harris 2000). This has been verified by use of x-radiography, allowing radicle emergence to be observed without disturbing the test. It was observed that the bound root was caused by the emerging radicle re-entering the seed, thus causing root growth termination in many seedlings. We found that if the seed were planted flat, the radicle never became bound in the seed coat. This phenomenon once observed allowed modification of testing procedures to prevent the bound root occurrence.

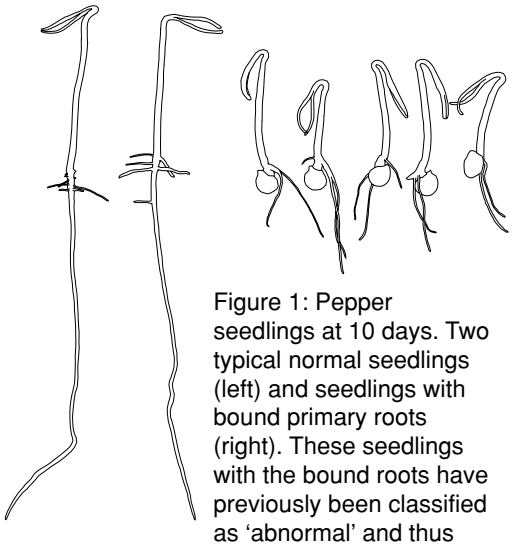

Figure 1: Pepper seedlings at 10 days. Two typical normal seedlings (left) and seedlings with bound primary roots (right). These seedlings with the bound roots have previously been classified as 'abnormal' and thus considered non-germinable.

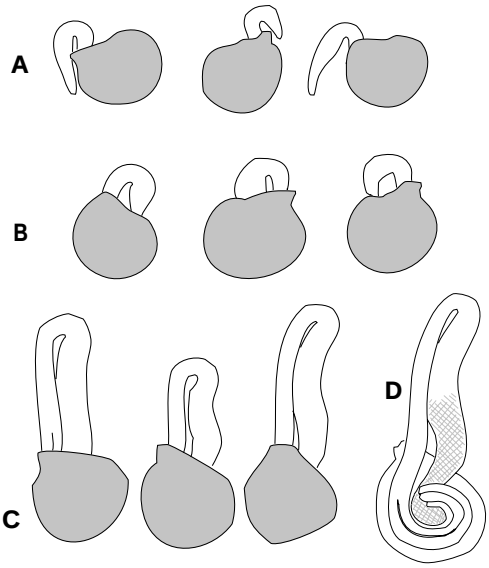

Figure 2: Germinating seeds with emerging radicles. (A) typical radicle emergence at three days, (B) radicles which have re-entered the seed coat after emergence, (C) developing seedlings with root and hypocotyl elongation after radicle re-entering, (D) a section view of seedling showing the bound root and cotyledons not yet emerged.

This study has increased uniformity between seed testing laboratories. CDFA test data have been used to support changes in the standardized pepper seed testing rules of the Association of Official Seed Analysts (AOSA), and documentation is now being prepared for support of changing the International Seed Testing Association (ISTA) rules and procedures.

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Examining the Accuracy of Scientific Descriptions for Caryopses of Johnsongrass and Sudangrass

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The importance of accurately identifying Johnsongrass seeds in agricultural seed cannot be over emphasized. Contamination of a 250 g laboratory sample by only one Johnsongrass seed is cause enough for the entire seed lot to be rejected from the California Seed Certification Program and prohibit its movement into many California counties.

California Crop Improvement Association (CCIA) seed analysts were concerned this year by the significant number of atypical Sudangrass seeds they were finding. The atypical seeds were small and possessed many Johnsongrass seed characteristics. They requested the assistance of the PPDC Seed Laboratory in a project that would identify these seeds and the plants they may produce.

Sorghum halepense (L.) Pers. has many common names, however, the most widely used is Johnsongrass. Johnsongrass is a perennial grass native to the Mediterranean area, purposefully introduced into California as a once desirable pasture and hay crop. The plant spreads by seeds (spikelets, caryopses) and creeping underground stems (rhizomes). The plant is a prolific seed producer, and seeds are the principal means of dissemination. The seeds can remain viable in the ground for over two years, making eradication of the plants extremely difficult. The plant's rhizomes are thick and fleshy with many scars where the leaves would be attached (nodes). These rhizomes present a special problem in that routine cultivation practices chop up the rhizomes into many reproductive units, each with the capability of producing a new plant. When recovering from frost or drought conditions, Johnsongrass can develop high levels of hydrocyanic acid, rendering it toxic to grazing livestock (Fuller and McClintock 1986). Many countries have declared Johnsongrass a troublesome weed; in United States its destructiveness has become so alarming that the federal government has taken action to bring it under control (Holm, et al. 1977). Today, the plant is well established in California as an aggressive weed, not only on cultivated land but in many disturbed areas below 2,600 feet.

Sorghum x *drummondii* (Nees ex Steud.) Millsp. & Chase, is commonly referred to as Sudangrass, an annual grass that also spreads by seeds (spikelets, caryopses) but does not produce rhizomes. Introduced into the United States from Africa for pasture, green chop, silage, and hay, it can yield as many as five cuttings in one year when grown in a warm climate.

Johnsongrass crosses with Sudangrass to produce a fertile hybrid. The plant is a perennial, similar in general aspects to Johnsongrass but with a less extensive and aggressive rhizome system.

The close relationship between Johnsongrass and Sudangrass leads to a pronounced similarity in their caryopses. In an effort to prevent the spread of Johnsongrass via contaminated seed lots, the United States Department of Agriculture produced two publications on the identification of Johnsongrass and Sudangrass caryopses: Bulletin No. 406, "Distinguishing Characters of the Seeds of Sudangrass and Johnsongrass" (Hillman 1916); and "Identification of Crop and Weed Seeds," Agriculture Handbook No. 219 (Musil 1963). These two publications describe the differences between Johnsongrass and Sudangrass caryopses, with Johnsongrass having smaller caryopses (2-3mm in length), as opposed to the larger Sudangrass caryopses (3.3-4.5mm in length).

Two lots of certified Sudangrass from CCIA were selected for this project. Laboratory samples of 250 g were obtained from the two seed lots, and all caryopses less than 3 mm in length were removed.

Eight small caryopses were removed from the first sample and six from the second sample. All the small caryopses were examined; only caryopses that fit all the characters stated in the USDA papers were classified as Johnsongrass. Two caryopses from each sample matched all the criteria for Johnsongrass caryopses. The four caryopses were planted at Orchard Park greenhouse (University of California, Davis), along with control samples of Johnsongrass and Sudangrass. The plants were allowed to grow for 124 days. The Johnsongrass controls produced plants with large extensive fleshy rhizomes mixed in with the roots, and the Sudangrass controls produced plants with only a fibrous root system, no rhizomes.

Johnsongrass, unknown, Sudangrass caryopsis

Of the four small caryopses identified as Johnsongrass, three produced plants. None of the three plants produced rhizomes. The small caryopses that match the USDA descriptions for Johnsongrass were Sudangrass.

Conclusion: The USDA descriptions for the identification of Johnsongrass and Sudangrass caryopses cannot be used to positively identify all small Sudangrass caryopses. One economical solution to this problem would be to clean out all Johnsongrass/Sudangrass caryopses less that 3 mm in length from seed lots before testing. There is still value in these descriptions, and it may be advisable to classify small Johnsongrass/Sudangrass caryopses found in seed samples that match all the characters of Johnsongrass as Johnsongrass to help prevent the spread of this troublesome weed.

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Johnsongrass rhizomes and root system

Sudangrass fibrous root system

Seed Technologist Training

Jim Effenberger, Marian Stephenson, Deborah Meyer, Paul Peterson, Elaine Harris

Seeds are the propagules and reservoirs of plant germplasm that farmers rely upon. Seed technologists must possess an array of skills and knowledge in the areas of purity and germination testing, seed vigor and genetic purity tests. The job of the seed technologist is to quantify the seed lot quality. These laboratory analyses serve as the basis for seed trade and thus the exchange of millions of dollars in seed

Figure 1. Examples of training materials used at seed workshops.

sales. Seed testing standardization is key to the success of the seed industry. Conducting seed workshops and providing individualized training for seed technologists is one part of the PPDC Seed Lab mission, with the goal of seed testing standardization among laboratories.

As part of this program we have developed numerous training materials for our annual workshops (Fig. 1). This year the California Seed Analysts and Seed Researchers Annual Workshop was held at Sakata Seed America, Morgan Hill, CA. PPDC scientists provided the bulk of the program with topics on identification of 37 species of Poaceae (grass family), 32 species of Polygonaceae (knotweed family) and the new Federal noxious weed species, seedling evaluation of the Asteraceae (sunflower family), Apiaceae (carrot family), Fabaceae (legume family) and Brassicaceae (mustard family).

This year the Society of Commercial Seed Technologists (SCST) published the *Seed Technologist Training Manual*, a comprehensive manual of seed testing principles, which has received international acclaim (Fig. 2). The manual contains over 450 pages, 150 color photographs and 735 drawings of seeds, presented in 15 chapters, authored by the most prominent seed technologists and researchers in the field. Two of our scientists were invited to contribute chapters on basic botany for seed technologists, seed identification and seed herbarium development and purity testing.

A popular resource on the CDFA website is the **Encycloweedia** - notes on the identification, biology, and management of plants defined as noxious weeds by California Law. Produced by CDFA's Integrated Pest Management Branch and the UC Cooperative Extension, the **Encycloweedia** is maintained by CDFA staff. Seed Laboratory scientists, who have proposed the addition of close-up photographs of seeds and fruits to the photo gallery, collaborated in photographing the propagules of the plants included in the website and worked with Julie Garvin (formerly of IPM), to see that appropriate images were incorporated in the **Encycloweedia** (Fig. 3).

Seed Laboratory scientists conducted individualized tutorial programs for persons training to become Registered Seed Technologists (RST) within the SCST. Under the training requirements for the RST, certification program individuals must complete numerous hours of practical training under the tutelage of a seed scientist outside of their own laboratory.

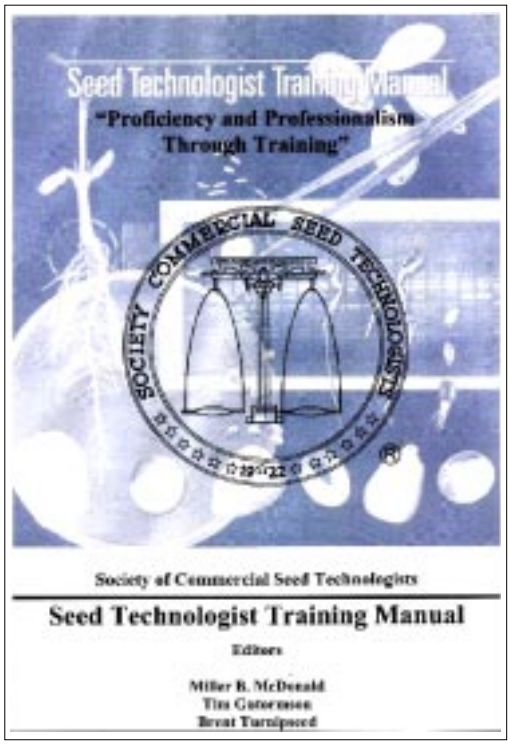
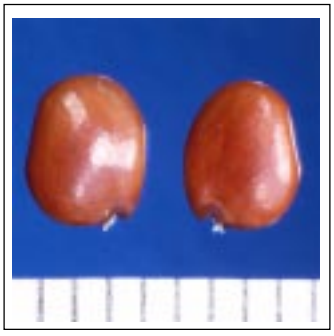


Figure 2. Seed Technologist Training Manual.



Seeds of
Spartium junceum L.

Milled seed of
Tribulus terrestris L.

Figure 3. Examples of noxious weed seed photos included in the CDFA Encycloweedia.

Development of More Efficient Seed Laboratory Testing Methods

Deborah J. Lionakis Meyer

Many of the seed laboratory testing methods used today were developed and standardized worldwide in the early 20th century. Use of these methods is required by various federal, state or provincial seed laws. Unfortunately, many of these laboratory testing procedures are very laborious and time consuming. In the modern era of the global seed market, rapid laboratory analysis of seed lot quality is essential. Today's economy also requires many laboratories to do more tests with a reduced labor force.

In the CDFA Seed Laboratory one research focus has been to seek more efficient ways of testing seed in the laboratory that will accurately express the quality of the seed in the marketplace. This type of research is just the first step toward changing a century of standardize practice. Gaining acceptance of the more efficient procedures by the seed testing community, the seed industry and by regulatory officials can be difficult. The following is an example of work recently completed that was successful in achieving acceptance in North America.

Figure 1. Type-A florets with attached structures equal to or longer than the fertile floret, and fertile florets with attached glumes and pedicel (S = sterile floret, F = fertile floret, G= glume, R = rachilla, P = pedicel).

Figure 2. Type-B florets with attached structures not extending to the tip of the fertile florets (S = sterile floret, F = fertile floret).

A study was conducted by the Association of Official Seed Analysts (AOSA) Research Subcommittee on Purity Testing, which compared the AOSA purity testing method for hard fescue (*Festuca brevipilia* R. Tracey) and sheep fescue (*F. ovina* L.) and three other more efficient methods. Ten government and commercial laboratories participated in the study, and as chairperson of the subcommittee, I served as the project leader. In these species, several florets, both fertile and sterile are attached together on the mature plant. During post-harvest seed conditioning, these structures are broken apart to make the seed flow better through planting equipment. According to the AOSA Rules for Testing Seeds, the standard by which all seed is tested in North America, seed units of hard and sheep fescue include single fertile florets and caryopses. In the laboratory, seed analysts are required to detect and remove all structures attached to the rachilla and base of the fertile floret. This is a tedious and time consuming process used to determine the amount of inert matter in a seed lot. The purpose of this study was to evaluate potential application of less laborious purity testing methods for hard and sheep fescue that are already in used for other grass species.

Figure 3. Inert matter recovered from Type-A florets (left) and Type-B florets (right).

The florets in this study were segregated into two groups: Type-A florets with various types of large attachments (Fig. 1) and Type-B florets with small attachments (Fig. 2). Figure 3 shows the

inert matter removed from each of these types of florets. For each species, eight seed lots were randomly selected from a pool of commercial lots. Sub-samples of each lot were randomly distributed to 10 laboratories with experience in testing hard and sheep fescue. The four testing methods used included:

Method 1 = AOSA method, in which all attached structures on Type-A and Type-B florets were removed and classified as inert matter or fertile florets.

Method 2 = sterile florets and glumes attached to Type-A florets were removed and classified as inert matter, attached fertile florets were separated, and Type-B florets were kept intact. This is the AOSA method for testing tall fescue (*Festuca arundinacea*) and meadow fescue (*F. pratensis*).

Method 3 = the amount of inert matter attached to Type-A florets was estimated based on a constant factor derived from the percentage of fertile florets (pure seed) in the Type-A florets. Both the Type-A and Type-B florets were kept intact. The intact Type-A florets are referred to as Multiple Seed Units (MSU) by various seed testing organizations. The constant factor for each species was based on the mean percent pure seed in the MSU for all samples tested for each species. This method provides an estimate of the percent inert matter attached to the MSU and is the AOSA method for testing red fescue (*Festuca rubra*).

Method 4 = International Seed Testing Association (ISTA) method, where the MSU are considered pure seed and no estimation of inert matter in the MSU is made. This method has not been deemed acceptable by the grass seed industry in North America.

Data for each species were analyzed separately. Weights of Type-A and Type-B florets, weight of inert matter detached from the fertile florets in the Type-A and Type-B floret groups and the time required to remove the structures were also recorded. Although the data showed there was significant variation in the results due to factors such as lab, lot, and method for the samples tested, Methods 2 and 3 produced nearly identical results (Table 1). Both of these methods produced results of approximately 0.2% higher

TABLE 1. Mean, Standard Deviation (SD), Coefficient of Variation (CV) across all laboratories and lots by method.

Species		Method 1	Method 2	Method3	Method 4
Hard Fescue	Mean	97.93	98.07	98.06	98.34
	SD	1.40	1.42	1.45	1.48
	CV	1.43	1.45	1.48	1.50
Sheep Fescue	Mean	96.73	96.90	96.89	97.54
	SD	1.15	1.15	1.20	1.17
	CV	1.19	1.19	1.24	1.20

pure seed than Method 1 (AOSA Method) for both species, an amount well within the allowable AOSA statistical tolerance. As expected, Method 4 (ISTA Method) deviated the most from Method 1, but results were within AOSA tolerance. All four methods had similar within-method variation.

As part of the method comparison, the study measured laboratory performance by examining bias, precision and accuracy values for each laboratory and method. Bias is a measure of average deviation from the mean and is an indicator of systematic error (a score of zero indicates no bias). Precision is a measure of laboratory consistency, the closer the precision value is to zero the more consistent the laboratory's performance across all lots tested. Accuracy values are a combination of bias and precision;

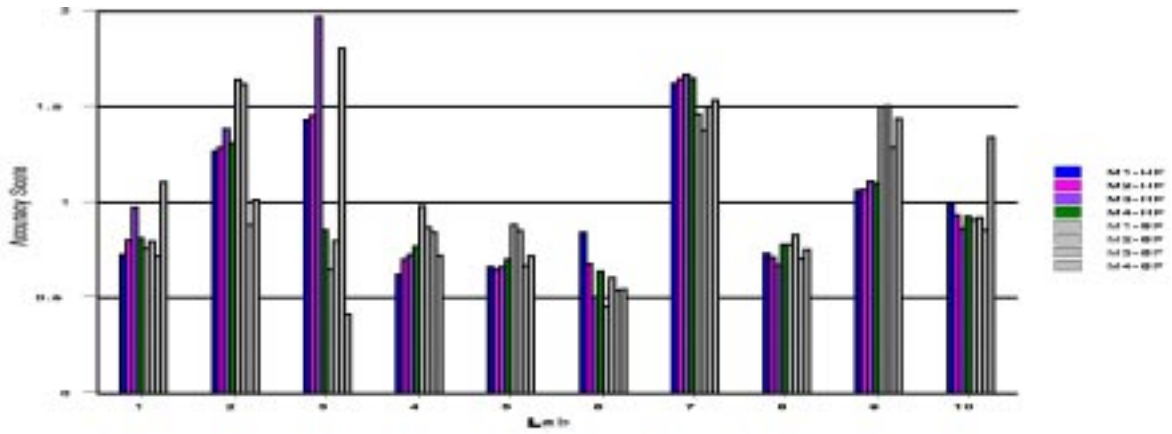


Figure 4. Accuracy values as a combination of bias and precision for each laboratory and method across all lots tested. (HF = hard fescue, SF = sheep fescue, M1 = Method 1, M2 = Method 2, M3 = Method 3, M4 = Method 4)

low values indicate a laboratory is consistently near the overall mean of all lots. Increasing accuracy values indicate a laboratory has a bias in one direction, is inconsistent, or both. Although, for the majority of labs involved, use of one or more of the alternative methods produced more consistent results across all lots than Method 1; laboratory accuracy for the majority of labs was better using Method 1 (Fig. 4).

Method 3 (factor method) showed marked timesaving (35% hard fescue, 41% sheep fescue) over Method 1(AOSA Method) and required less physical manipulation of the seed units (Fig. 5). Method 3 was formally adopted by the AOSA and became the standardized method for testing hard fescue and sheep fescue in North America, effective October 1, 2001. For details of this study see Meyer (2001).

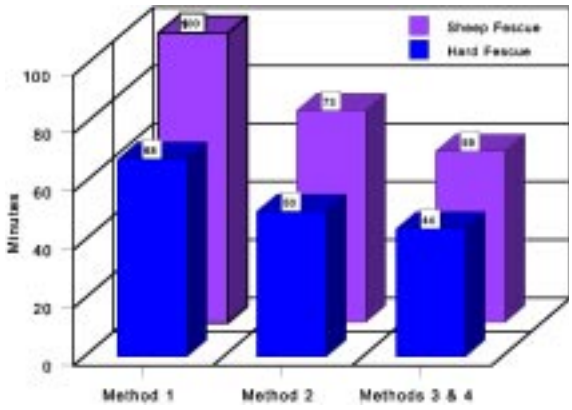


Figure 5. Mean time (minutes required to complete physical separation of the purity components for each method and species.

Acknowledgments

Thanks to Larry Prentice (Nebraska Crop Improvement Association) and the University of Nebraska Biometrics Laboratory for their assistance with the statistical analysis and the following seed laboratories for their participation: California State Seed Laboratory (Jim Effenberger), Canadian Food Inspection Agency, Central Seed Laboratory (Doug Ashton), Georgia Department of Agriculture's Atlanta Seed Laboratory (Aida Galarza, Lonnita André, and Diane Hembree), Growmark, Inc. (Stewart Oliver), Maryland State Seed Laboratory (Jennifer Miller), Michigan State Seed Laboratory (David Johnston), Pennsylvania State Seed Laboratory (William Cook), Utah State Seed Laboratory (Stan Akagi), and Washington State Seed Laboratory (Victor Shaul). Appreciation is also expressed to Stewart Oliver and Victor Shaul for providing the seed for the project and Johanna Naughton (CDFA) for sample preparation.

Reference Cited

Meyer, D.J.L. 2001. Comparison of four purity methods for *Festuca brevipila* R. Tracey and *F. ovina* L. Seed Technology 23:35-49.

Observations on the Laboratory Germination and Seedling Growth of a Hybrid Globe Artichoke (*Cynara cardunculus cardunculus*) cv. Kiss of Burgundy

Paul S. Peterson and Elaine Harris

Standardized seed germination tests of globe artichoke (*Cynara cardunculus cardunculus*) using the procedures of the Association of Official Seed Analysts (AOSA) and the International Seed Testing Association (ISTA) both specify testing at alternating 20˚/30˚C (8 hr./16 hr.) with a test duration of 21 days. Some globe artichoke cultivars recently tested in the CDFA seed laboratory, such as cv. Kiss of Burgundy, suggest that the standardized testing regime may not be suitable for all artichoke cultivars. There are 140 cultivars recognized and 40 in common use today. Some of our laboratory observations indicate that test temperature and test duration may require testing rule changes. Globe artichoke is considered a cool climate vegetable. It is not surprising to see the germination and seedlings of the cultivar Kiss of Burgundy (Figures 1 & 2) favoring the cooler constant 20˚C test temperature. The higher recommended temperature of 20˚/30˚C, which is used in testing, does not appear optimal. Also observed in this cultivar, test duration at both 20˚C and 20˚/30˚C was necessarily extended past 21 days by another 2-3 weeks for complete germination.

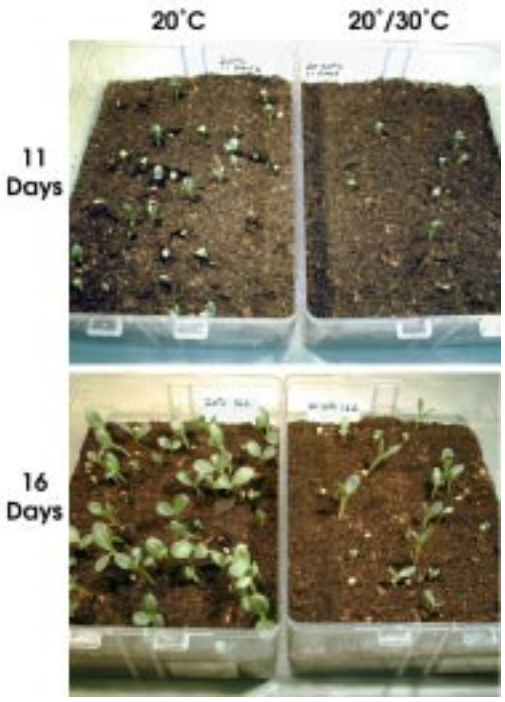


Fig. 1 Seedlings of the hybrid cultivar, Kiss of Burgundy grown at a contant 20° and alternating 20°/30°C (apparently, cultivar favors lower test temperature).

Currently, a controlled germination study is being made using three cultivars, Kiss of Burgundy, Imperial Star, and Green Globe. Because research literature indicates that light may inhibit germination of artichoke, particularly at higher temperatures, and that excess moisture may also inhibit artichoke germination, all of these factors are being examined. Moisture may be inhibiting germination through the production of a mucilage layer on the outer surface of the seed at higher temperatures. Others have demonstrated when seeds are returned to cooler temperatures germination will resume. These three cultivars are being germinated at 20˚C, 15˚/25˚C and 20˚C/30˚C. At each of these temperatures, both light and dark illumination are used. The germination percent, germination rate, and observations of seedling morphology, such as root and hypocotyl lengths, are being recorded.

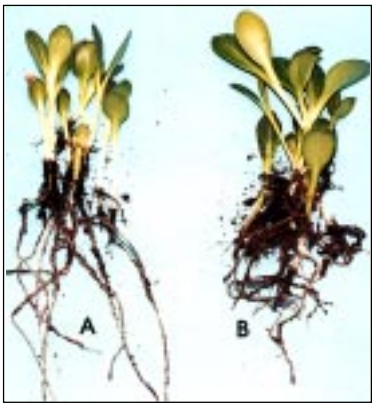


Fig. 2. Globe Artichoke seedlings cv. Kiss of Burgundy at two test temperatures (A, constant 20°C and B, alternating 20°/30°C). Seedling elongation is greater at higher temperature, but at 20°C seedlings have more rubust shoot and root growth.

While our investigation continues, we recommend that laboratories testing globe artichoke use both 20˚C and 20˚/30˚ temperatures in the dark. Preliminary data indicate that some modifications in the standardized testing procedures will be needed.

National Ryegrass Grow-out Study

Marian Stephenson, Evelyn Ramos, and Jason Miller

Although seeds of both annual (*Lolium multiflorum* Lam.) and perennial (*Lolium perenne* L.) ryegrass are marketed, the two readily hybridize, and botanists debate whether two species actually exist. It is impossible to distinguish individual florets (seed units) of the two using morphologic characters. This has created a problem for seed certification.
Highly undesirable in turfgrass is the occurrence of plants having the morphologic characteristics associated with "annual" ryegrass (commonly referred to as "big and ugly" but more appropriately described by differences in leaf width, color and growth habit, Fig. 1).

Fig. 1. Plants ranged from those exhibiting the full complement of morphologic characters associated with annual ryegrass (left: wider blades, lighter green color, elongated stems and head formation within 42 days) to those recognized as perennial (right: narrower, darker blades; shorter stems; and no head formation within the test period).

Scientists at Oregon State University (OSU) Seed Laboratory and the USDA-National Forage Production Research Station at Corvallis, OR, recently proposed that a grow-out test for ryegrass be used as a supplement to the laboratory test currently accepted by the Federal Seed Act and the Association of Official Seed Analysts (AOSA). The Association of American Seed Control Officials (AASCO) passed a resolution supporting purity labeling of ryegrass seed lots on the basis of the grow-out test. The PPDC Seed Laboratory agreed to participate in a national study to evaluate the protocol.

Four samples of 'unknown' ryegrass and a control sample of annual ryegrass were provided by OSU. Each sample was tested according to AOSA Rules for germination and fluorescence. All seedlings exhibiting fluorescence; 20 randomly selected non-fluorescing seedlings from each sample and 20 seedlings from the annual control sample were transplanted into planting mix. The transplanted seedlings were placed in a Percival Model RE-16 Growth Chamber and held at a constant 25° C temperature under full light (270 mmol m-2 s-1 or higher). Flats were watered as needed with half-strength Hoagland's No. 1 nutrient solution.

Plants that flowered or resembled the annual control plants (wide blades, light color, elongated stems, reddish-purple-tinged leaf sheath bases) within 42 days were classified as annual types. Plants with narrower blades, darker color and shorter stature that did not flower within 42 days were classified as perennial. The numbers of annual type ryegrass plants found in the samples using the standard laboratory fluorescence test and the grow-out test are compared in Fig. 2.

These data and those from eight other laboratories will be analyzed to provide a basis for standardization of test procedures to measure the variability in results among laboratories and to study the correlations between the fluorescence test, the grow-out test and varietal fluorescence levels.

At one time it was thought that annual ryegrass seedling roots exude a substance, annuloline, under prescribed laboratory test conditions and that perennial ryegrass seedling roots did not. Test procedures that utilized the fluorescence of annuloline under ultraviolet light were established and accepted as the best available method for differentiating the two species by AOSA and by the U.S. Department of Agriculture (USDA).

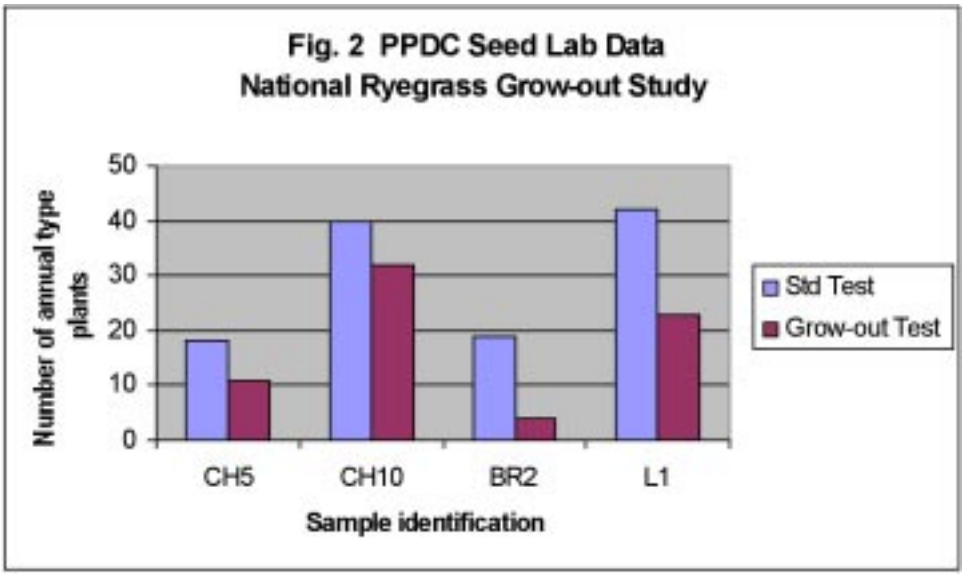
Even at that time, scientists were reporting that some varieties of perennial ryegrass produced as much as 20% fluorescing seedlings. Some varieties of annual ryegrass were reported to exhibit only 64-87% fluorescence (rather than the expected 100%).

Subsequently the formula AOSA and USDA applied to fluorescence test data to calculate the percentage of perennial ryegrass was revised when it was established that the variety 'Linn' perennial ryegrass produces as much as 5% fluorescent seedlings.

Most recently (1991), varieties of annual ryegrass with less than 100% fluorescence and varieties of perennial exhibiting ANY fluorescence must be characterized by breeders. The Association of Official Seed Certifying Agencies (AOSCA), Grass Variety Review Board receives and evaluates supporting material and maintains a list of varieties and their fluorescence levels. New formulas were constructed that take into account the fluorescence level inherent to each variety. Thus, fluorescence observed in a test is not a direct indicator of the presence of annual ryegrass; rather, the level of fluorescence is compared mathematically to the level established for the variety being tested and tolerances are applied.

Scientists at OSU and USDA believe that the fluorescence test is not an adequate indicator of ryegrass growth habit due to gene flow between the species. Over the past 10 years they have accumulated data that indicate that environmental factors, time of year the seed is tested, variety of ryegrass, and sampling variation all have significant effects on the fluorescence test results.

OSU has submitted a proposal to AOSA to adopt the ryegrass grow-out protocol as a supplemental test. It is anticipated that the grow-out test is an interim test; work is ongoing to develop a DNA-based test to distinguish between annual and perennial ryegrass. Development of a procedure that can be applied more precisely in many more laboratories is highly desirable.



A Study on the Effect of Malformed Cotyledons of Polyploid Watermelon (*Citrullus lanatus* L.) on Germination and Seedling Growth.

Paul S. Peterson

Early seedling growth in the family Cucurbitaceae depends on the photosynthetic activity cotyledons, which expand and become leaf-like. Malformed cotyledons are common in triploid and tetraploid seed of watermelon (*Citrullus lanatus* L.). These polyploids are used in the breeding and production of seedless watermelons. This malformity may be caused by mutations resulting in defective cotyledon formation during embryo development or seed maturation. In seed testing, the cotyledon structures are evaluated and must meet certain criteria for the seed to be classified as germinable. Evaluation problems of malformed cotyledons has resulted in non-uniformity in seed germination test results among different seed laboratories.

Currently the effect of cotyledon malformities on germination is being studied in the CDFA seed laboratory. This study will help establish improved seedling evaluation guidelines for use in seed laboratories. In this study, X-radiographs (see Figure 1) of polyploid seed are made, and individual seeds are numbered and germinated to determine embryo defects on seedling development as shown in Figure 2. Figure 3 shows exposed embryos, normal and with a malformity. Malformities include fused cotyledons, cotyledons folded, cotyledons orientated incorrectly in the seed, misshapened cotyledons, incomplete cotyledon filling of the seed cavity. In this study, seed weight and other data are being collected which will also help seed processing to clean out some of the defective seed by weight or seed density.

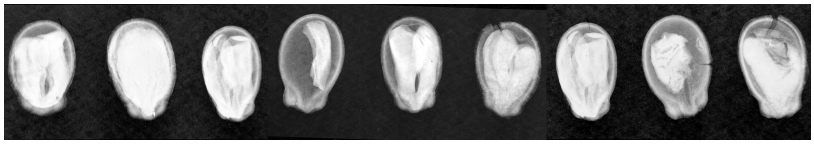


Figure 1: X-radiograph of a triploid watermelon seed [one seed appearing normal (second from the right) while the others show various degrees of malformity and maturity].

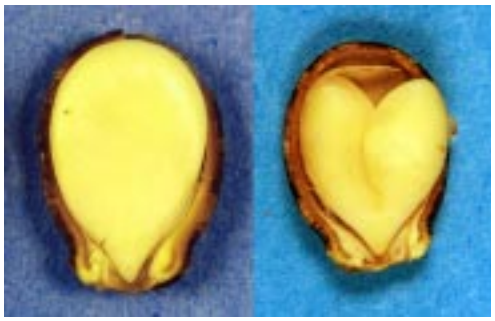


Figure 2: (above) Two watermelon seeds with embryos exposed [a completely normal embryo (left seed) and an embryo with incorrect orientation of the cotyledons (right seed)].

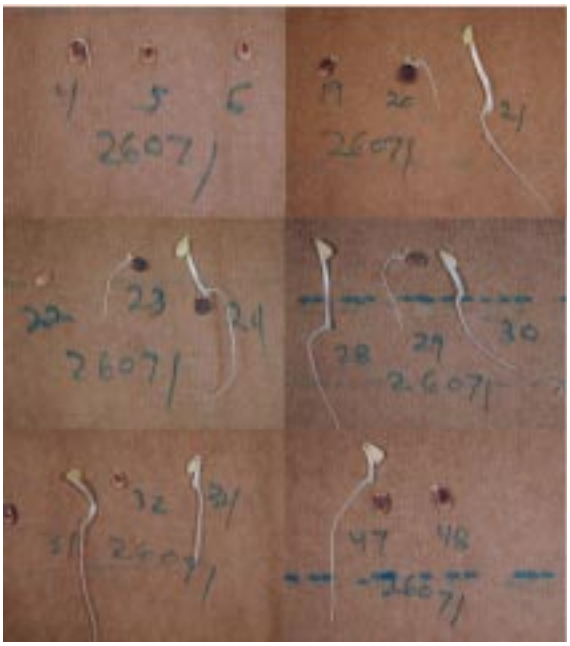


Figure 3: (right) An example of some seedlings produced during germination of a poor seedless watermelon sample such as shown in the X-ray above.

Plum Pox Potyvirus Survey

Dennis E. Mayhew, Kathy Kosta, Terra Irving

Plum pox potyvirus, one of the most serious diseases of stone fruits in Europe, was found in Pennsylvania in 1999. This was the first time this disease was detected in North America. The following year, PPV was found in Canada. Susceptible hosts of PPV include peaches, apricots, plums, nectarines, almonds, and sweet and tart cherries. Symptoms vary from color break in flowers to ring and mosaic patterns in leaves to severe fruit markings and distortion. There is a reduced yield of marketable fruit, sometimes severe depending on variety of fruit and which strain of virus.

Because of the devastating nature of this disease and the potential economic impact on the Prunus industry in the United States, the U. S. Department of Agriculture initiated a nationwide survey for PPV in states where Prunus is grown commercially. The virology laboratory of the Plant Pest Diagnostics Branch has completed the second year of testing of California trees using an ELISA (Enzyme Linked ImmunoSorbent Assay) test developed in Spain. In 2000, 46,479 trees were tested comprised of 33,453 mother trees registered with the California Nursery Program and 13,026 trees used as bud wood sources for the production of common stock. In 2001, an additional 52,386 trees were tested which represented the remainder of registered trees, trees used to produce common nursery stock, and a limited number of commercial trees. All tests for PPV proved negative, and there is no evidence that this virus occurs in California. The 2002 survey will focus primarily on commercial trees.

The number of trees tested each year is limited, because higher growing temperatures reduce the titer of the virus in plant tissue and makes detection difficult. Survey and testing is now limited to the months of April through June when average daytime temperatures do not exceed 85° F for more than a few days. New testing procedures are being developed which will allow year-round testing of trees.

Symptoms of plum pox potyvirus in peach
(photo courtesy of USDA)

Detection and Analysis of Potato Virus Y Necrotic Strain in California

Tongyan Tian, Dennis Mayhew, Joyce Tuttle, and Terra Irving

Potato virus Y (PVY) is an economically important plant virus on potato and has a wide host range including potato and tobacco. Based on symptoms caused on potato and tobacco, PVY can be divided into two major groups, PVY common stain (PVY-o) and PVY necrotic strain (PVY-n). Although PVY-o is distributed world wide, PVY-n historically has been limited to Europe and Eastern Canada. For PVY-n, therefore, beside causing virus disease in potato directly, it is also a quarantined virus affecting potato export and import.

In June of 2001, we received plant samples from potato fields located in New Cuyama of Santa Barbara County. Initial ELISA tests indicated that the samples from two of the four plots contained PYV-n. More carefully designed experiments immediately followed to confirm the identity of these virus collections.

We first used a series of ELISA tests using monoclonal antibodies against PVY-o and PYV-n separately. Those were monoclonal antibodies "1F5" and "295.5" against PVY-n and Mab2 against PVY-o.

Table 1. Results of ELISA tests for PVY-n detection.

	295.5**	1F5**	MAb2**	Remarks
Lot 202	1/11*	0/11	0/11	+ for PVY-n
Lot 194	5/14	4/14	0/14	+ for PVY-n
Lot 232	0/15	0/15	0/15	- for PYV-n
Lot 233	0/11	0/11	0/11	- For PVY-n

* Each sample was composed of tomato 10 leaflets, and expressed as # of positive over # of total samples.

** Monoclonal antibodies.

Our ELISA showed that a total of 6 sample groups were positive to at least one monoclonal antibody against PVY-n. All the samples positive for "1F5" ELISA were also positive for "295.5," but no samples reacted with "Mab2" for PVY-o. These data indicated that the potato plants in New Cuyama were infected with the quarantined virus, PVY-n. The differences in ELISA tests between "1F5" and "295.5" were likely due to the sensitivity of the antibodies. Because PVY-n is a quarantine virus, detection of this virus has significant implications on California potato. Additional measures were taken to be certain that our detection was not due to false positive of the ELISA tests.

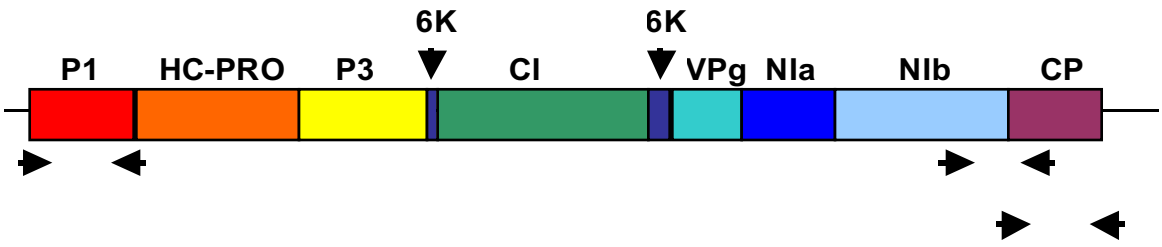


Figure 1. Schematic representation of PVY genome. Arrow pairs underneath represent Oligo-primers in the corresponding regions for polymerase chain reaction analysis.

We have utilized reverse transcriptase polymerase chain reaction (RT-PCR) to amplify the nucleotide sequence of the suspected PVY-n detected in New Cuyama. Using designed specific primers against several regions of PVY-n genomic RNA (Figure 1), we were able to amplify DNA products from those sample groups positive in ELISA test but not from those negative in ELISA. When the nucleotide sequence of the suspected PVY-n was compared with the PVY-n and PVY-o sequences in the GenBank, it clearly showed that this virus belongs to the PVY-n group. In addition, our sequence comparison also reviewed that the PVY-n detected in New Cuyama is likely different from those previously detected in North America. In fact, this virus is most similar to PVY-n isolates reported in Europe, including isolates of potato tuber necrotic stain (PVY-ntn), the viruses in the PVY-n group but also causing severe damages on potato crops.

The potato crop in New Cuyama was the first generation of potato propagated from the seed potatoes previously grown in Idaho and Nebraska. During our communication with the virologists in other states, we learned that PVY-n has been also detected in several other states. By sharing the virus RNA sequences determined by this laboratory, we have learned that the virus detected in New Cuyama is almost identical to one of the virus isolates detected in Idaho, suggesting that the two collections might have been introduced from the same source.

We have made a lot of progress in the diagnostics of PVY-n. However, both ELISA and nuclear acid sequence comparison are indirect tests for the biological properties. Many questions remain unanswered regarding this virus. The potato field in New Cuyama was well isolated from all the major agricultural production areas in California. Following our initial detection of PVY-n, we conducted a survey on potential PVY alternative hosts in the area. However, no PVY-n was detected. Our data and the information provided to us suggest the virus is not established in the area. Our focuses, therefore, are to prevent introduction of the virus and collaborate with virologists outside of state agencies to analyze biological properties of the virus.

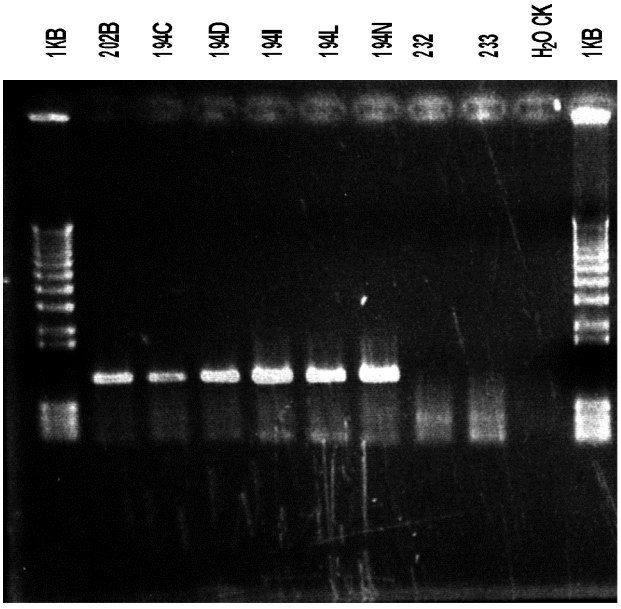


Figure 2. Agarose gel electrophoresis analysis of the RT-PCR products from the potato samples. Labels on the top indicate the samples from different lots. All the samples positive for PVY-n in ELISA tests were also positive in RT-PCR. No RT-PCR products were detected for the samples negative in ELISA and no PCR product was detected for water control. The 1 KB DNA ladder (1 KB) was used as the size markers.

Detection of Beet Curly Top Virus Using Polymerase Chain Reaction (PCR)

Joyce Tuttle, Tongyan Tian, Dennis Mayhew, Bryce Falk*, Paul Guy*, and Terra Irving
(*Department of Plant Pathology, University of California, Davis, CA.)

Beet curly top virus (BCTV) belongs to a group of plant DNA viruses, Geminivirus, and is transmitted by the beet leafhopper, *Circulifer tenellus*. In order to prevent potential economic loss due to BCTV, the state of California conducts BCTV control programs to reduce the beet leafhopper population and evaluate the spread of BCTV in field crops.

In the year 2001, an increase of BCTV incidence in the fields was observed. Laboratory diagnostics for BCTV was requested from the BCTV control program. Oligo-primers were designed by the virologists, Bryce Falk and Paul Guy of UC Davis, based on all the BCTV isolates in the literature. DNA extraction protocol was adapted from the UC Davis virology laboratory, and multiple dilutions were tested for efficiencies of PCR amplification.

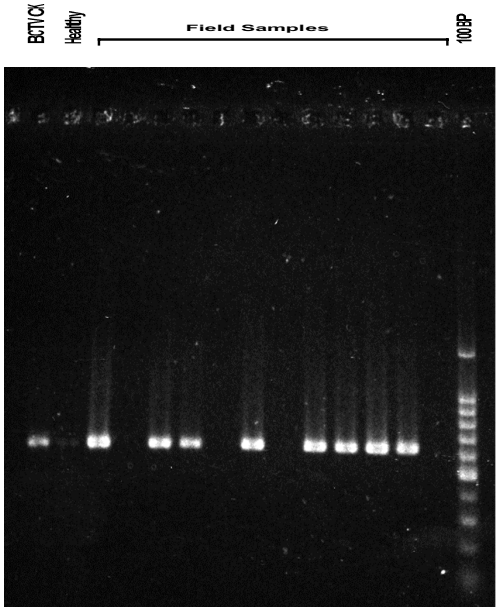


Figure 1. Agarose gel electrophoresis analysis of BCTV DNAs amplified using PCR and BCTV specific oligo-primers. Control DNA templates were extracted from BCTV infected plant (BCTV CK) and healthy plant (Healthy). The 100 BP DNA ladder (100 BP) was used as the size makers.

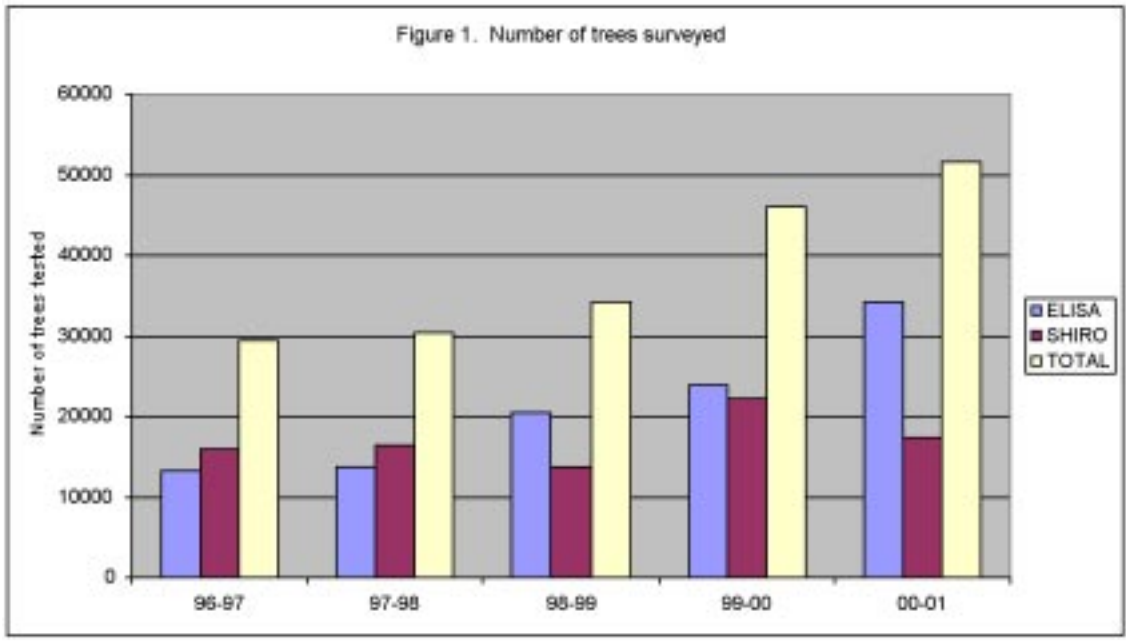
After initial tests, DNA dilution was standardized at 10 fold for all field samples. Stringent PCR detections were performed with DNA controls from BCTV positive and healthy plants along with each panel of field samples. The presence of the amplified DNA of ca. 630 base pairs was interpreted as BCTV positive and the absence of the DNA as negative. A total of 142 plant samples was analyzed at the virology laboratory for BCTV and 60 samples were tested positive for BCTV. In some cases, a majority of the samples from a field collection was tested positive for BCTV.

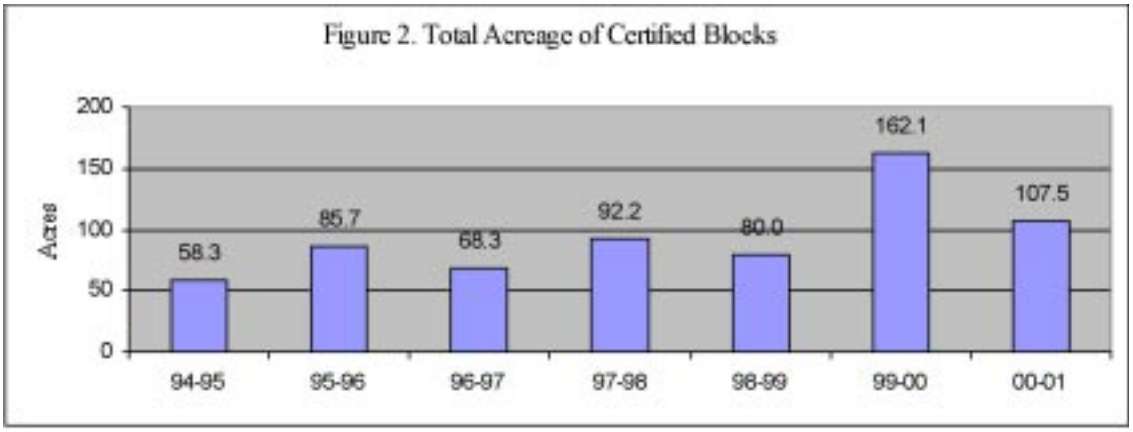
BCTV is a common virus in the central valley region of California. ELISA test is still not available at this time for BCTV detections and PCR or hybridization based detection has proven useful for reliable diagnostics of the virus. During the last season, we have successfully implemented the PCR techniques for BCTV detection in the PPDC virology laboratory and improved our sample processing efficiency from DNA extraction to the final agarose gel electrophoresis analysis. We are looking forward to participating in future plant virus detection using nuclear acid based technologies in the laboratory.

Annual Survey of Deciduous Fruit and Nut trees for Viruses

Y. Zhang, U. Kodira, and A. Noguchi

California Fruit Tree, Nut Tree, and Grapevine Improvement Advisory Board (IAB) has been allocating funds to test various fruit trees, nut trees, and grapevines for viruses for the participant nurseries. In the Registration and Certification program for fruit and nut trees, all trees in a Registered mother block, registered scion block as well as seed block are tested for viruses annually. The testing for viruses may be done by indexing in Shirofugen cherry or by Enzyme-Linked Immunosorbent Assay (ELISA). The trees are tested for Prunus necrotic ring spot virus (PNRSV) and Prune dwarf virus (PDV) by indexing at least once every five years and by ELISA for these viruses in the other four years. This cyclic schedule was implemented starting in the 2000-year season. These tested trees may be used as a source of certified propagative material in the year following testing. Due to increasing demands to participate in the Registration and Certification Program (R&C) by nurseries, the number of trees and total acreage have been expanding over the years (Figs. 1 & 2)

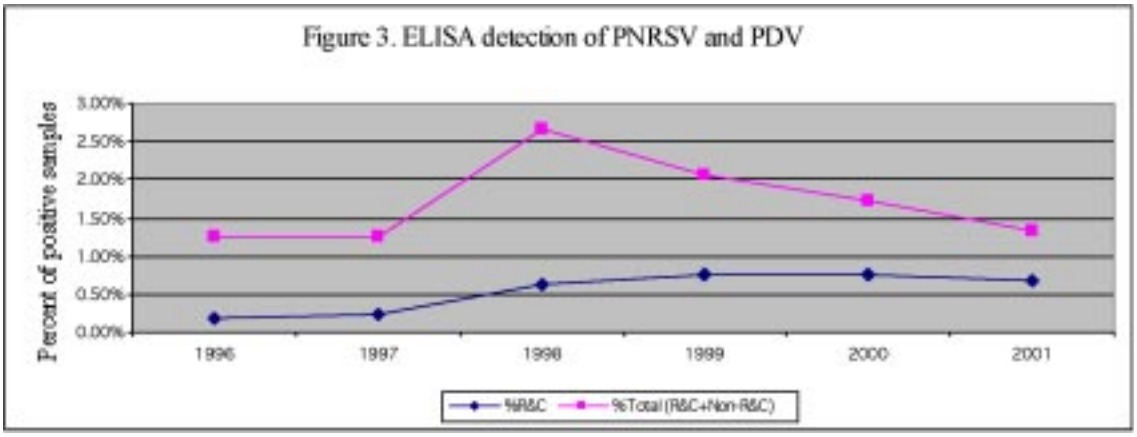




The total number of trees tested in 2000-01 was 51,686 (34,235 by ELISA and 17,451 by Shirofugen indexing). This number is 5,673 trees (12.3%) more than the previous year (1999-00). There were 26,410 R&C samples and 7,825 service samples tested by ELISA. Of the total 34,235 samples tested by ELISA, 587 (1.72%) were positive for the two ilarviruses. However, only 200 (0.76%) of the R&C sample were positive for the same viruses. Among the non-R&C samples, 387 (4.95%) were positive for viruses (Figure 3). Another 24 samples (0.14%) tested positive for the viruses by Shirofugen indexing.

In the year 2001-02, a total of 45,914 stone fruit nursery tree samples were tested by ELISA for the two ilarviruses, which is 11,679 (34.1%) more than the previous year (2000-01). Of the total 45,914 stone fruit tree samples (36,078 R&C samples and 9,836 service samples) tested, 614 samples (1.34%) were tested positive for the two ilarviruses. There were only 247 (0.68%) of the R&C samples tested positive for the same viruses while 367 (3.73%) of the service samples were tested positive, which is a much higher infection by the viruses (Fig. 3). The tests were done on samples from 19 participating nurseries. The stone fruit trees being tested included: peach, nectarine, almond, apricot, cherry, plum, and prune.

Figure 3. ELISA detection of PNRSV and PDV



The ELISA test is being performed using polyclonal and monoclonal antibodies in a combined format for both Prunus necrotic ring spot virus and Prune dwarf virus to reduce cost. The ELISA plates are first coated with a mixture of polyclonal antibodies of both PNRSV and PDV, followed by incubation with sample extracts. Monoclonal antibodies of both PNRSV and PDV, along with alkaline phosphatase, conjugated goat, anti-mouse antibody were then added to react with viral antigen. Positive samples are detected by addition of substrate for alkaline phosphatase and color change. A positive sample is scored by the detection of either one of the two viruses or both.

Acknowledgements: This project is supported by California Fruit Tree, Nut Tree, and Grapevine Improvement Advisory Board, Pest exclusion biologists, and participating nurseries.

Peach Fruit and Foliage Problem

James Smith

I participated in a multi-agency, fact-finding meeting regarding a "disease" problem that affected thousands of acres of peach trees in several northern counties during the summer. Approximately 18 individuals from Agricultural Commissioner offices, University Extension representatives, California Department of Pesticide Regulations and others attended. Considerable controversy had arisen over the cause of a widespread problem of necrotic spots occurring on peach foliage and fruit. Differences of opinion as to the cause of the small, necrotic spots on fruit and foliage were the impetus for the meeting. Numerous samples of affected foliage had been collected and analyzed by various county biologists and plant pathologists. Some peach growers thought the cause was failure of their fungicide applications used to protect against the 'shot hole' disease caused by the fungus *Wilsonomyces carpophilus*.

The large amount of rice production in the area requires extensive applications of herbicides to control weed growth in these rice plantings. Herbicides used in previous years often caused damage when it drifted offsite entering into the fruit tree plantings. Therefore, a new herbicide called Clincher® was used this season and aerially applied to thousands of acres of rice fields for weed control. It was expected that this material would eliminate phytotoxicity problems caused by older herbicides normally applied for weed control. Unfortunately, unexpected phytotoxicity and considerable drift of the material from surrounding rice fields into adjacent peach orchards was estimated to have caused the loss of 1,000 tons of peach fruit.

Initially there were differences of opinion as to the cause, but after considerable discussion and the similar opinions obtained from most of the participants, it was apparent that county personnel, extension specialists and I were all in general agreement that Clincher® was the probable cause. No signs of a pathogen involvement were found. Although late in the season by this time, the University Extension specialists were requested to spray some test trees with Clincher®. They responded that they already had planned to try to correlate the apparent phytotoxicity of the chemical by spraying some peach trees. It was agreed that due to the lateness of the growing season the peach test tree foliage may have already become "hardened off" too much to respond with herbicide symptoms so this test trial may be inconclusive this year.

Pierce's Disease Control and Monitoring

B. Hill, D. Opgenorth, and R. Randhawa

Pierce's Disease, a serious vineyard disease caused by the bacterium *Xylella fastidiosa*, kills grapevines. The bacterium multiplies and spreads in the xylem tissue of the vine until bacterial aggregations are formed that block the movement of xylem sap (water), causing symptoms that resemble water stress. Diseased vines become non-productive and may die in as little as 1 or 2 years after infection. The disease has been present in California since at least the 1880's. While the disease has at times caused epidemic losses in some southern desert and San Joaquin Valley areas, it has not been a major threat to the economic viability of viticulture in most of California.

The disease is vectored by sharpshooter leafhopper insects (Homoptera, Cicadellidae) that feed on xylem sap and spread the disease from plant to plant. California's three most important vector insects have been the blue-green sharpshooter (*Graphocephala atropunctata*), the green sharpshooter (*Draeculadephala minerva*), and the redheaded sharpshooter (*Carneocephala fulgida*). In the past with the exception of some traditional hotspots, the disease has infected a low percentage of grapevines, and the loss of vines has been within the range of economic viability. In the early 1990's a new insect vector appeared in California, the glassy-winged sharpshooter (*Homalodisca coagulata*). The glassy-winged sharpshooter (GWSS) is a much larger insect than the traditional vectors, and it's feeding and flight behavior make it a much more effective vector for the disease. Where GWSS is present, the spread of the disease can be so rapid and extensive that epidemic losses can soon render large areas of vineyards non-viable.

In the mid 1990s, GWSS appeared in the citrus groves adjacent to vineyards in the Temecula area of southern California. The population of GWSS increased rapidly, and by 1999 an epidemic of Pierce's Disease led to the loss of more than 300 acres of vineyards. Recognizing the unprecedented potential threat to California's wine and table grape industries, the California Department of Food and Agriculture, with funding from state, industry, and federal sources, established the Pierce's Disease Control Project. The goal of the project is to stop the spread of GWSS into the non-infested areas of California, thereby, preventing further epidemic outbreaks of Pierce's Disease, and to find ways to control GWSS and Pierce's Disease in already infested areas.

The Pierce's Disease Control Project has five central elements: 1.) Prevention of the spread of GWSS into new areas by regulating shipments of host plants and plant materials; 2.) Statewide survey and detection to monitor GWSS infestations and populations through trapping and visual inspection; 3.) Rapid response to develop and implement work plans to treat and, if possible, to eradicate new infestations; 4.) Outreach to growers and the public to raise the awareness about Pierce's Disease and respond to concerns; 5.) Research to develop solutions to reduce or, if possible, eliminate the risk of Pierce's Disease.

Following the epidemic in Temecula, populations of GWSS appeared and began to increase in parts of Kern County near Bakersfield near General Beal Road. Again, GWSS attained large numbers in citrus groves and then moved into adjacent vineyards in the grape season. In the winter of 2000-2001 the Pierce's Disease Control Project created the General Beal Pilot Study Project to research and determine ways to control GWSS and the resultant epidemic of Pierce's Disease. An important aspect of this project was to monitor the percentage of vines infected with the disease and to determine the increase in Pierce's Disease infections in those vineyards infested with GWSS.

To determine the rates of Pierce's Disease infections in vineyards both infested and not infested with GWSS, about 5000 total acres were tested - about 4000 acres non-infested with GWSS and 1000 acres of GWSS infested vineyards. The GWSS infested vineyards were in the General Beal study area in Kern County. The vineyards not infested with GWSS were in southern Tulare and northern Kern counties. In the GWSS infested areas, two tablegrape varieties most susceptible to Pierce's Disease (red globe and flame seedless) were tested, because these were the varieties most likely to exhibit recognizable symptoms within one year of inoculation. In the vineyards non-infested with GWSS, the majority were also red globe and flame seedless, but other varieties were tested as well. In all the acres tested people walked the rows in the vineyards and mapped and marked the vines that exhibited symptoms. Samples were collected from those vines showing strong symptoms and were sent to the CDFA's Plant Pest Diagnostics Branch laboratory in Sacramento to be tested. In most cases about 60–80% of the samples submitted to the laboratory were found to be positive for Pierce's Disease by ELISA testing. In all about 3,500 vines were sampled and tested by the laboratory.

In the vineyards not infested with GWSS, there was a fairly uniform incidence of Pierce's disease, about one vine in 10,000 occurring randomly. In some localized traditional hot spot areas the incidence was as high as 1%, or one vine per 100. Such sampling and testing of the GWSS infested vineyards in the General Beal study area was not done in the past, but the experience of local experts and Pierce's Disease advisors was that before the arrival of GWSS the incidence was probably about the same, or one vine in 10,000. There did not appear to have been traditional hot spots in the study area, and Pierce's Disease was not considered to be a problem in Kern County. In the GWSS infested study area, the incidence of Pierce's Disease has increased dramatically. The incidence of Pierce's disease in the vineyards in this area now range from 5% to as much as 30%, sharply up from the historical levels of one in 10,000. This increase in infection rates has occurred over the last two years, since 1999.

Report For The Year 2001

Dan Opgenorth

This year, a considerable amount of time was spent working with the Glassy Winged Sharp Shooter (GWSS) and Nursery Services Branch to provide diagnostic results for detection of Pierce's Disease (*Xylella fastidiosa*). Work for Nursery Services resulted from a quarantine on California grown grape stock imposed by the Oregon Dept. of Ag. We established good working relationships with the Kern County Ag. Comm. Office, the Farm Advisors in Kern and Tulare counties. and contacts from private industry. Based on our laboratory results, field survey methods have been developed and improved. The need to document the exact vineyard sites provides an expedient way to take a representative sample; proper handling of samples was emphasized. This was done to facilitate survey and grid mapping by the GWSS Branch using our PDR record determinations.

ELISA testing using specific antibodies has been the basic diagnostic technique used for the determination of Pierce's Disease. Support staff are trained to use the DAS HP technique as produced by Agdia. While very practical, the method is slow, not especially sensitive and must be done in a laboratory environment. This season I worked with Agdia on three occasions to field-test a rapid Dip Stick antibody test. On the first occasion samples were collected from a Red Globe vineyard near Exeter and processed by maceration in small plastic bags. Results were apparent in 10-20 min and seemed to be consistent with previous testing from this block of vines. On the second and third occasions the results were not as definitive and suffered from what I would consider to be false positives when compared with other laboratory results (ELISA and PCR). The test was not consistent enough to use in the field where it may have been helpful to train survey scouts in disease identification. Since white and table grape varieties grown in the southern San Joaquin Valley do not manifest definitive leaf symptoms, I am hopeful that Agdia will continue to work on this methodology. The Dip Stick assay could be extremely valuable for future survey work.

PCR technology using the Hopkins primers (Rst31/33) was originally done by our lab as a more sensitive method of testing for *Xylella*. The original CTAB extraction method for total DNA used several grams of tissue and large glass centrifuge tubes. A mini prep was developed using 0.5 g of tissue and disposable microtubes. This made it possible to process tissue using our ball mill (KLECKO) apparatus. To further expedite DNA extraction, suggestions were made to Richard Garcia, who is the engineer at KELCKO, to design similar equipment for single use polyethylene tubes. A 32-tube machine was built, tested and seemed to perform well.

Other techniques were also explored to facilitate DNA extraction for PCR assays. The Fast-Prep system was useful, but woody tissue is not easily processed unless diced into 2 mm portions. This unit has some plastic parts that were not very durable. An immuno-capture technique was acquired from the UCD laboratory of Bruce Kirkpatrick. This was modified to utilize microfuge tubes and our KLECO processor. However, in comparing notes with workers at Agri-Analysis (private laboratory), we both realized that this method was more laborious and no more sensitive than the original CTAB extraction procedure. Membrane blot technology from D Squared was investigated and found to be laborious, confusing and not particularly definitive.

The Chemicon Corp. of Temecula Calif. has developed a very sensitive Oligo PCR assay. Patrick Schneider, a Chemicon scientist, spent several days working with us to test the assay. The procedure is unique in that target DNA is blotted on a cellulose card, purified on the card and a 1 mm piece then used as a DNA sample to perform the PCR. Amplified DNA is then hybridized to a target DNA sequence immobilized on a 96 well plate. An aviden-biotin HP system is used to detect the product. The test

preformed well with great sensitivity. Even single insects (GWSS) were determined to be infested with *Xylella*. Later work, however, did show that false positives could be problematic due to nonspecific binding of primer-dimer products. This was apparent when the primers were not previously aliquoted and stored at –20 C. We also suggested that the mixture of primers be packaged and stored separately in the future.

The most interesting and innovative diagnostic technology was Real Time PCR. Dr. Norman Schaad (USDA-ARS) was a visiting scientist in the laboratory for one week. Dr. Schaad is developing this diagnostic system for several regulatory bacterial diseases. He developed primers for *Xylella* and using the Smart Cycler helped to do an early season detection survey in Tulare, Kern and Napa counties. We were clearly able to detect the disease in the trunk of vines in April when the sap was running or from wood macerates taken from dormant vines. Real Time PCR is extremely sensitive and showed perhaps greater sensitivity when coupled with selective culture enrichment. This technology has great promise and application for rapid field and port of entry use in the detection of regulatory bacterial plant diseases such as Citrus Canker.

Using diagnostic methods the CDFA laboratory provided support to do field research concerning the spread of Pierce's disease in the vineyards. With the help of Gisela Wittenborn (Sunview Vineyards) and numerous field scouts, vine by vine surveys were done to determine where the disease could initially be found and if vine to vine spread was occurring. Preliminary information indicated that weedy fallow areas outside of vineyards could be initial sources of inoculum and that vine to vine spread was a later problem due to involvement of GWSS. Vineyards involved in these studies should continue to be monitored to follow disease progression on a yearly basis and to determine if removal of infected vines may have value in disease suppression.

Diseases incited by mycoplasma continued to be of importance this year. We are still receiving samples for Peach Yellow Leaf Roll from nursery mother trees. The techniques used to isolate mycoplasma DNA for PCR testing continue to be improved. Hopefully, new work will allow the use of more specific primers in the future. Late this year I received a call from Mike Davis (UCD Extension Plant Pathologist) concerning a Corn Stunt outbreak in Kings and Tulare counties. Testing to confirm the disease was required, and our CDFA diagnostic lab set up the assays to provide ELISA and PCR results. These results may be necessary for crop insurance compensations where the losses were 20 to 50%. Staff from the County Ag. Comm. Office were instructed to sample any fields that they believed to be involved. I expect that this will continue to be a problem and would hope to continue providing our diagnostic service to the counties and University in an effort to further investigate this disease problem..

The culture collection acquired from UC Berkeley continues to present an overwhelming amount of work. We continue to find contamination and dead cultures but also some viable and authentic isolates. The work is slow and tedious but will serve as a future resource. Presently, we have done extensive work on the *Agrobacterium* species and attempted to build our own BIOLOG database. I will expect to do more with this as time will allow next year. While BIOLOG offers a good initial means of characterization, other options need to be explored for confirmation purposes.

On December 6, 2000, The California Plant Disease Conference was held at the Diagnostic Center. This was the Ninth Conference with the goal of informing our county clients concerning special projects and other issues. Topics were presented on Plum Pox, Karnal Bunt, Sudden Oak Death, GWSS, Pierce's Disease, with updates in Entomology and Nematology. Biotechnology was the afternoon topic with presentations on the Permit Process, Transgenic Plants and Problems associated with GMO's. We had a sell-out crowd of over 80 participants, many of which arrived at the last moment.

Mycology and Seed Health Testing Section

Tim Tidwell, Diana Fogle, Allen Noguchi, Raj Randhawa

Sudden Oak Death (SOD) took center stage this year. PPDC pathologists served as part of the SOD task force, which includes scientists and biologists from the U.S.D.A. Forest Service (USFS), California Department of Forestry and Fire Protection (CDF), University of California, Davis and Berkeley(UCD & UCB), University of California Cooperative Extension (UCCE), the Agricultural Commissioners_ Offices, Cal Poly San Luis Obispo, and a host of other local municipalities and agencies, such as water departments, park departments, etc. The role of PPDC on the task force is primarily to provide diagnostics for samples originating from counties within the known area of SOD infestation and assistance in the mapping of their distribution. Although much attention has been focused on the staggering mortality of coastal range stands of Tan Oak (*Lithocarpus densiflorus*) and Coast Live Oak (*Quercus agrifolia*), CDFA must also pay special attention to the particular threat of SOD to the Nursery industry. In response to surveys involving large numbers of Rhododendrons and other susceptible nursery stock, PPDC Staff Mycologist Diana Fogle developed an innovative rapid diagnostic technique for identifying the SOD pathogen, *Phytophthora ramorum*, in infected Rhododendrons within 48 hours of receipt of the sample. In addition, a screening technique using serological methods was also developed for screening large numbers of foliar samples, to reduce the number to a manageable quantity of disease suspects for culturing when necessary. PPDC scientists also participated in a SOD laboratory workshop given at UCD.

Cotton Fusarium wilt pathogen (FOV). Australian cotton seed was imported this season for cattle feed, but because of concerns of a new strain of the FOV being introduced to California, seed health tests had to be conducted on all the seed lots entering California. In a cooperative effort with emeritus Professors James DeVay (UCD) and Shirley Smith (UCB), as well as UCCE specialist Michael Davis, the seed was tested and some were found to be infested with various species of *Fusarium*, including *F. oxysporum*, despite having been fumigated with methyl bromide. Testing is still in progress, but no FOV has been detected thus far.

Karnal Bunt pathogen, *Tilletia indica.* PPDC plant pathologists again traveled in rotating shifts down to eastern Riverside County to test wheat seed for the presence of the Karnal Bunt, as part of a cooperative project with the USDA to monitor and manage the distribution of this pathogen in California. After three consecutive years of no positive detections of the pathogen in the region, this year three fields - all adjacent and belonging to same grower - were heavily infested with bunted kernels of the disease. Because of these new detections, monitoring will likely continue next season.

Lettuce Mosaic Virus (LMV). Formal CDFA approval was granted to four private California laboratories to test lettuce seed for LMV to meet local phytosanitary requirements. The program is administered by the Seed Health Testing Staff of PPDC. To gain CDFA approval, laboratories must meet several criteria, including an annual site inspection, successfully testing several blind samples of lettuce seed, and evaluation of laboratory protocols and personnel. It is anticipated that more formalized accreditation of private laboratories for this and other seed health tests will follow in the near future. PPDC is likely to play a significant role in this laboratory accreditation process.

Three new diseases. Projects involving the identification of specimens collected from Pest Detection disease surveys for three new diseases, groundsel rust (*Puccinia lagenophorae*) and Daylily rust (*Puccinia hemerocalidis*), and Rice Bakanae (*Fusarium fujikori*) resulted in the substantial expansion of the known distributions of these three diseases. These diseases are currently under further study by a number of scientists from the USDA, Purdue University, and UCCE.

Pine pitch canker pathogen, *Fusarium circinatum.* A potential disaster involving the pitch canker of pine disease was averted as a result of a cooperative project involving S.L.O. Co. Agriculture Department, CDFA PPDC and UCD plant pathology department. A nurseryman received long leaf pine seed at his nursery in San Luis Obispo Co, located in a part of the state that has already been hit hard by the devastating pine disease. The pine seeds had been produced in a pitch canker infested area in the southeastern U.S. and brought into California to grow as seedlings to be shipped back to the east coast for reforestation. As a result of seed health tests and seedling tests performed at the PPDC plant pathology lab, the pine pitch canker pathogen, *Fusarium circinatum*, was isolated from both the seedlings and the seed. Since nearly all the inoculum is located on the seed surface, the Commissioner's office subsequently required the nurseryman to receive only fungicide treated seed, which eliminates the inoculum and substantially reduces risk. Subsequent seed tests demonstrated that the risk was virtually eliminated by using only treated seed. Thus, the introduction of a new isolate of the pitch canker pathogen from the southeastern U.S.A. was prevented.

Training in seed health testing procedures for Rice Blast Disease was provided at the PPDC for scientists from the California Crop Improvement Association.

Diseases new to California were detected by the Mycology lab. These included a new leaf spot disease of Columbine (*Stemphylium lancipes*); Vinca Rust (*Puccinia vincae*) and Acoris leafspot (*Ascochyta acori*).

Mycological expertise of staff mycologist Diana Fogle served California's diverse Agriculture industry in new and creative ways. UCCE extension pathologist Steven Koike, working on the characterization of a new lettuce disease, turned to PPDC for help in identifying the pathogen, as well as other mycological assistance. Likewise, California based seed companies doing research on seed-borne pathogens have also turned to PPDC for similar mycological help. Cleaning up, reviving, and manipulating fungal cultures for taxonomic studies and for pathogenicity inoculum preparation, etc. requires considerable mycological skill and expertise, involving such techniques as single sporing, light microscopy, selective media development, etc. These were new ways in which PPDC found to serve both the seed industry and lettuce industry in California.